



## An Early Molecular Biomarker Study in Pre-eclampsia

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**Abstract:** Preeclampsia (PE), a hypertension condition associated with pregnancy that manifests after 20 weeks of pregnancy, may be brought on by faulty placental development and poses a serious risk to both the mother and the fetus. The disease can be detected only once the symptoms arise, there is a need for a biomarker, which makes early detection of the disease possible, and proper management of the disease can be done at an early stage of pregnancy. Even though many studies were done globally regarding the role of microRNAs as molecular biomarkers in pre-eclampsia, no literature showed the effect of microRNA in preeclampsia in the Indian population. The aim of the study was to find an early molecular marker that can detect the disease at an early stage, even before the symptoms arise. Placental MicroRNAs were studied using Next-generation Sequencing of 60 Preeclamptic groups (30 Early Onset, 30 Late onset preeclampsia), and 30 control groups were selected; miRNA profiling was done by Illumina sequencing, and the quantifier did downstream analysis for identification, quantification, and expression profiling. pl, script, and miRDeep2.pl, script. Total RNAs were extracted from placental tissues and cells by TRIzol reagent and purified. The relative expression of miR-483-5p in tissues or cells was determined: micro-RNA 483-5p was expressed in significant quantity in Early Onset preeclamptic placental samples compared to Late Onset and normal samples. miR 483-5p was analyzed, and the gene targets were found using computational methods. From this study, it was found that microRNA 483-5p can be used as an early biomarker for the identification of preeclampsia.

**Keywords:** microRNA, Biomarker, Next-generation Sequencing, Pre-eclampsia, RT-PCR

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## I. INTRODUCTION

The prevalence of preeclampsia, a multisystemic pregnancy-associated condition that develops after 20 weeks of pregnancy, is 5-8% globally and 8-10% nationally.<sup>1</sup> The World Health Organization (WHO) estimates that hypertension disorders alone are to blame for 70,000 maternal deaths and 500,000 neonatal deaths each year globally.<sup>2-4</sup> The condition manifests as de novo hypertension, with symptoms including 140 mmHg systolic, 90 mmHg diastolic, and, in extreme cases, 160 mmHg and 110 mmHg.<sup>5,6</sup> In addition to the symptom, other conditions, such as HELLP syndrome, IUGR, proteinuria, edema, etc., are usually present. Doppler analysis, proteinuria assessment, and estimated blood pressure are usually employed to diagnose the disorder. The condition is also accompanied by neurological symptoms like headaches, stomach aches, and blurred vision. Preeclampsia can be classified as Early Onset Preeclampsia (34 weeks) and Late-Onset Preeclampsia (>34 weeks) based on gestational age.<sup>7</sup> Moreover, early-onset preeclampsia is significantly more likely to be linked to a higher risk of cardiovascular illness, indicating a significant role in the maternal constitution<sup>8</sup>. The placenta is thought to be the disease's primary cause. Hence the only treatment now in use is to deliver the placenta.

### 2.1 Pathogenesis of Pre-Eclampsia

Transforming the cytotrophoblast (CTB) into extravillous trophoblast (EVT) is a critical stage for placental implantation. EVT formed at the terminals of the anchoring villi invades the endometrium and maternal spiral arteries. These arteries migrate away from the placenta, changing the mother's immune reactions and structure into high-volume conduits that encourage uteroplacental blood flow. Narrow resistance arteries become wide conduit channels in the maternal spiral arterioles<sup>9</sup>. At the end of the first trimester (10–12 weeks), this initial occurrence lasts until 18–20 weeks into the pregnancy. The mechanism by which the cytotrophoblasts transform from an epithelial phenotype to an endothelial phenotype during this vascular invasion is known as pseudovasculogenesis. There are several similarities between the epithelial-to-mesenchymal transition (EMT), which occurs during the development of an embryo, the healing of wounds, and the metastasis of cancer. This process involves several transcription factors, growth factors, and cytokines, including VE-cadherin and alpha v beta-3 integrins<sup>10</sup>. Because they invade spiral arterioles shallowly and leave them as small caliber resistance vessels, invasive cytotrophoblasts in pre-eclampsia do not successfully transition from an epithelial to an endothelial phenotype, which leads to poor uteroplacental circulation and reduces placental perfusion. Pre-eclampsia results in hypoxia in the intervillous area of the placenta, which may lead to oxidative tissue stress, increase placental apoptosis and necrosis and cause endothelial dysfunction and exaggerated inflammatory response. Antiangiogenic factors like soluble forms-like tyrosine kinase-1 sIg-I and soluble endoglin are released more often as angiogenic factors necessary for placenta growth are diminished in the circulation. As a result, altered endothelial expression of coagulation factors causes coagulopathy, increased vascular permeability causes proteinuria, and endothelial regulation of vascular development is lost.<sup>11</sup> Studies have revealed that pre-eclamptic women have higher serum levels of interferon gamma-inducible protein (IP-10), monocyte chemotactic protein (MCP-1), intercellular adhesion molecule (ICAM-1), and vascular cell adhesion molecule (VCAM-1), and lower

levels of interleukin-10, suggesting that the placenta may also be a source of circulating inflammatory cytokines<sup>12</sup>.

### 2.2 Cellular interactions

Both soluble molecules, such as chemokines, cytokines, steroid and protein hormones, as well as substances delivered by extracellular vesicles, are used by the STB to communicate with the maternal immune system.<sup>13</sup> The three primary vesicle types covered by the term "EV" are exosomes, microvesicles (also known as ectosomes and microparticles), and apoptotic bodies. The smallest vesicle type, exosomes, range in size from 50 to 150 nm and are produced constitutively by the endocytic pathway in multi-vesicular bodies (MVB)<sup>14</sup>. These MVBs enable exosomes to be loaded with a specific cargo before being released into the extracellular environment through fusing with the plasma membrane and exocytosis. Microvesicles (100 nm-1 m) are promptly released from the cell in response to variables such as cellular activation or stress that elevate intracellular calcium levels and cause cytoskeletal remodeling via surface contacts such as protein or lipid ligand-receptor binding, fusion, and release of their contents into the target cell's cytoplasm, and finally endocytosis and subsequent fusion with endosomes. It is believed that EVs communicate with their target cells. EV transports RNAs, lipids, and proteins (such as mRNA, miRNA, vault RNA, and tRNAs). The STB is the primary placental source of EV and may act as an important signaling channel between the mother and fetus, boosting maternal physiology to allow the presence and meet the needs of the developing fetus.<sup>15,16</sup> If the condition could be evaluated before the symptoms appear, the patient may be managed and given the correct therapy. A growing body of research suggests that several molecules, such as endoglin, placental growth factor (PIGF), and the soluble form of VEGF receptor (sFlt-1), which are linked to abnormal angiogenesis and involved in blood vessel formation, may be used as early maternal serum indicators for PE<sup>17</sup>. These proteins are only discovered in the bloodstream after the damage has already been done. Hence, a biomarker is required to identify the disease before it manifests.

### 2.3 Angiogenic Markers

Angiogenesis, or the growth of new blood vessels from existing ones, is a crucial stage in the vascularization of the placenta. Pro- and antiangiogenic elements work synergistically to mediate it. VEGF and placental growth factors (PIGF) are angiogenic markers, and VEGFR-1 and 2 receptors are associated with antiangiogenic factors.<sup>18</sup>

### 2.4 The Placental Protein Biomarkers

The placental protein biomarkers include the following: ACTH (adrenocorticotropic hormone), ADAM12 (a disintegrin and metalloprotease 12), ADMA (asymmetric dimethyl arginine), CRH (corticotropin-releasing hormone), hCG (human chorionic gonadotropin), MAP (mean arterial pressure), NO (nitric oxide), pp-13 (placental protein-13), PIGF (placental growth factor) (vascular endothelial growth factor).<sup>19</sup>

### 2.5 Molecular Basis and Markers

Pre-pathogenic eclampsia's mechanisms begin in the first few weeks of pregnancy when the molecular plane experiences most modifications. Molecular groups from maternal blood may have the potential for early prediction because the

placenta is crucial in the onset and development of the illness. Its significance is demonstrated by the fact that the only effective treatment for pre-eclampsia is the delivery of the fetus and placenta. Indicators may be observed in the maternal circulation as proteins, hormones, metabolic products, or DNA<sup>21</sup>. It has been demonstrated that molecular biomarkers such as microRNAs are useful for the early identification of preeclampsia. A short endogenous non-coding RNA called a microRNA (miRNA or miR) is important for post-transcriptional control. By binding to the target miRNA's 3'-region, it is one of the most important regulators of the gene expression of protein-coding genes and contributes significantly to post-transcriptional gene regulation.<sup>21</sup> One may impact several cellular pathways, and the fact that it is released into the bloodstream from native cells suggests that it participates in whole-body cell-to-cell communication. It has been demonstrated that many miRNAs highly expressed in the placenta regulate cell motility, invasion, apoptosis, and proliferation<sup>22</sup>. Several miRNAs were also particularly expressed by the placenta and released exocytotically into the mother's blood. The placenta of preeclampsia patients had abnormal miRNA expression, according to a comparison between them and women who were pregnant normally<sup>23</sup>. The importance of miRNA-regulated gene expression in the pathophysiology of PE and the physiological process of placental development has been the subject of numerous studies.<sup>24</sup> Although technological advancements over the last ten years have increased our understanding of miRNAs' functions during pregnancy, many unanswered questions remain. It is in part because there needs to be more research that fully examines how prenatal environmental exposures and pregnancy-related problems affect miRNAs. Additionally, there are gaps in our understanding of the function of miRNAs during pregnancy due to the use of inconsistent techniques in research. Few Indian studies explain the role of microRNAs current study focuses on the occurrence of placental microRNAs, which are differentially expressed in preeclamptic conditions compared to normal pregnancy. MicroRNA, which shows significant expression, is selected. Its differential expression is studied in preeclamptic samples compared to normal and was checked for usefulness as a biomarker. miRNA target was found using target scan software.

## 2. MATERIALS AND METHODS

This multicentric study in the obstetrics and gynecology department was carried out through cooperation between tertiary care hospitals like the Fernandez Hospital in Hyderabad, the Employees State Insurance Corporation, the Apollo Institute of Medical Sciences, and the Department of Molecular Genetics, Pathcare Labs, Red Cliff Labs. From January 2020 to March 2022, two years and three months were spent doing the study.

### 2.1 Ethics-Related Matters

The institutional ethics committee of the relevant institutes granted consent for this investigation (EC approval numbers: 008/09/2019/IEC/SMCH: ESIC-ESICMC/SNR/IEC-S101/12-2020: Fernandez -EC Reference No. 32 2020). The study's subjects provided their written, informed consent. According to the Helsinki Declaration - Ethical Principles for Medical Research Involving Human Subjects, we ensured the study complies with all applicable international ethical standards. Inclusion criteria: The study group involved 30 preeclamptic women and 30 normal healthy control women. Women of the

age group between 18-45 years were selected. Placental samples were collected after the diagnosis of PE was based on blood pressure with systolic  $\geq 140$  mmHg and diastolic  $\geq 90$  mmHg and proteinuria (ACOG2019) 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.

### 2.2 Isolation of Total RNA Containing miRNAs

Total RNA containing miRNAs is isolated from tissues by Trizol extraction. We have used Ambion's Nirvana miRNA isolation kit to isolate good-quality total RNA from placental tissue.<sup>25</sup>

### 2.3 Materials

The tissue of interest, fresh or stored in RNAlater (Qiagen, cat. no. 76106)

Trizol (Life Technologies, cat. no. 15596-026)

miRVana miRNA isolation kit (Ambion, cat. no. AM1560)

Bioanalyzer 2100 (Agilent) with nano or pico chip.

Samples with an RNA integrity number (RIN)  $> 8$  yield high-quality libraries.

RNA quality was confirmed using Agilent Bioanalyzer with a nano chip.

### 2.4 Construction of A Multiplex Mirna Library for Illumina

#### 2.4.1 Sequencing

#### 2.4.2 Rna Extraction

RNA is extracted from frozen tissue using manual methods of Trizol and LiCl. Total RNA quality and quantity are assessed on Agilent Bioanalyzer/TapeStation and Qubit Fluorometer. RNA samples with an RNA Integrity Number above seven (RIN $>7$ ) are considered for sRNA-Seq library preparation.<sup>26</sup> A miRNA library is made from each RNA sample by 3' adapter ligation, 5' RT primer annealing, 5' adapter ligation, reverse transcription, and PCR amplification. In addition, each miRNA library is amplified using a common forward PCR primer and a unique barcoded reverse primer.

### 2.4 Rna Isolation and Extraction

Total RNA was purified using NucleoSpin ® RNA Plus (Cat no: 740971), Takara. RNA sequencing was done by Illumina sequencing and generated files for downstream analysis with the miRDeep2. The quantifier does identification, quantification, and expression profiling. pl, script, and miRDeep2.pl, script. The real-time expression of miRNA was 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), dH<sub>2</sub>O, and relevant TaqMan probe (Applied Biosystems). miRNA protocol was run on an ABI PRISM 7900HT PCR system at the following settings: 95 °C, 10 min; followed by 95 °C, 15 s; 60 °C, 1 min 40 cycles.<sup>27-29</sup>

## 3. STATISTICAL ANALYSIS

The experimental data were expressed as mean  $\pm$  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 one-way ANOVA.  $P < 0.05$  was considered a statistically significant criterion. Each experiment runs at least three times. This study included patients aged 18-45 pregnant women with preeclampsia. The sample size was calculated based on the formula.

$$\text{Sample size} = 1.96^2 \sigma^2 / E^2$$

Where  $\sigma^2$  is the Std deviation, and E is the standard error <sup>30</sup>

#### 4. RESULTS

The expression of miRNA profiling by the NGS study showed the expression of miR483-5p, which was significantly expressed in preeclamptic samples.

**Table 1: Expression level of miR483-5p in EOPE, LOPE, and Control.**

S.no	hsa-miR 483-5P	EOPE	LOPE
1	0.0691	6.9825	1.1523
2	1.0552	8.2344	0.45355
3	0.12415	4.0987	2.9554
4	0.2775	3.7640	2.32007
5	1.6920	2.37847	1.3255
6	0.9465	9.4738	0.332525
7	0.2876	3.7485	0.33145
8	0.9874	7.2394	1.0447
9	0.8346	3.8764	2.0147
10	0.0947	9.8765	0.0104
11	1.9832	5.3378	2.8571
12	0.8456	2.3476	1.478
13	3.1298	3.7465	0.895
14	0.4839	8.4327	0.23855
15	0.9084	8.2345	0.2587
16	0.9234	4.1235	1.1475
17	0.1290	6.3402	0.1505
18	0.9344	2.9834	0.1785
19	0.98309	7.4332	1.9885
20	0.47633	4.8976	2.1785
21	0.98744	3.84654	2.0895
22	0.3098	5.90287	0.9885
23	1.0002	9.3484	1.447
24	0.8734	8.23465	0.5895
25	0.0233	7.3442	2.2585
26	0.9347	8.3345	3.3685
27	0.9876	6.5963	1.9455
28	0.2348	4.6725	2.1885
29	0.8946	7.234	1.2558
30	0.4467	7.4818	2.8588
Mean	0.7953	6.0848	1.4101
Std.Dev.	0.6416	2.2392	0.9655

Table-I: shows increased expression of miR 483-5P in Early Onset Preeclampsia as compared to Late-Onset PE and Control.

**Table-2 -F Statistics Result Details**

Source	SS	df	MS	
Between-groups	502.1119	2	251.0559	$F = 118.46873$
Within-groups	184.3682	87	2.1192	
Total	686.4801	89		

The f-ratio value is 118.46873. The p-value is < .00001.

**Table-3 Post Hoc Tukey HSD (beta)**

Pairwise Comparisons	HSD.05 = 0.8963	Q.05 = 3.3722	Q.01 = 4.2308
Control: EOPE	M1 = 0.80 M2 = 6.08	5.29	$Q = 19.90$ (p = .00000)

Control: LOPE	M1 = 0.80 M3 = 1.41	0.61	Q = 2.31 (p = .23625)
EOPE: LOPE	M2 = 6.08 M3 = 1.41	4.67	Q = 17.59 (p = .00000)

The result(Table-1) shows that miRNA 483-5p is expressed highly in Early onset samples compared to Control samples and Late Onset Preeclamptic Samples. When the expression of 483-5P is compared with Late-onset and control samples, the expression is higher in Late-onset preeclampsia. There is a 6-fold increase in the expression level of 483-5p in Early onset samples compared with control samples and 5-fold increase in the expression in early-onset samples compared with late-onset samples. Table-2 showws the F-statistics and p

value which shows significance value of less than 0.00001. Post Hoc Tukey HSD (beta)(Table-3) was conducted to find pairwise comparisons within the ANOVA data. Here is a significant difference between Control vs. EOPE and also EOPE Vs. LOPE. But no significance between Control Vs. LOPE. It shows that microRNA 483-5p is differentially expressed in three samples, and it is more significant between control and EOPE and between EOPE and LOPE.

**Table -4. Genes targeted by microRNA483-5p. Given a list of other miRNAs which target the same genes**

Genes Targeted	Micro RNAs
GFRA4(GDNF family receptor alpha-4	483-5p
SMAD2(SMAD family member2	MiRNA 483-5p,372-3p,520c-3p,135b-3p.
MAPK-1(Mitogen-Activated Protein Kinase I)	miRNA-483-5p,518c-5p,524-3p,518b.668-5p.
BCL2 -BCL2 apoptosis regulator.	181b-5p,494-5p,432-5p,431-3p
SOCs 3	483-5p
IGF2	483-5p

Note -the above table shows the genes targeted by microRNAs. The action of these miRNAs silences gene expression. This table shows that along with miRNA 483-5p, other microRNAs also target the same genes. MicroRNA 483-5p was chosen for the study due to the better expression levels obtained from the samples collected. MiR - 483-5p negatively regulates GFR $\alpha$  1–4 receptors, negatively affecting the embryo's development. The neurotrophic factors belonging to the GDNF (glial cell line-derived neurotrophic factor) family greatly benefit numerous populations of central and peripheral neurons. The receptors for these components are GDNF family receptor 1-4 (GFR-1) ligand-binding subunit and Ret tyrosine kinase complexes. GDNF receptors were highly expressed throughout embryonic development in the developing skin, bone, muscle, endocrine glands, urogenital, digestive, respiratory, and neurological system<sup>31</sup>. GDNF was also expressed in the urogenital system and developing limbs. Only a few mesenchyme/epithelial induction sites where GDNF factors and receptors are generated in the embryo include the kidney, tooth, and submandibular gland. The concept that GDNF components take part in inductive processes throughout embryonic development is supported by this pattern of expression <sup>32</sup>. Increased expression of miRNA483-5p results in the silence of the mRNA that codes for GFR1-4 receptors, which impairs the ability of GDNF proteins to function.<sup>33,34</sup> Another target of MiR 483-5P is Bcl2. Bcl2 is adversely regulated by miRNA 483-5p (b-cell sarcoma). The proapoptotic protein located in the outer membrane of mitochondria belongs to the Bcl-2 family of regulator proteins that control cell death (apoptosis) by either blocking (anti-apoptotic) or stimulating (pro-apoptotic) apoptosis.<sup>35</sup> Research showed that samples from preeclamptic patients exhibited less Bcl-2 expression. Thus, in preeclamptic women's placental beds, elevated trophoblast apoptosis brought on by excessive apoptotic activity precludes trophoblast invasion into the spiral artery.<sup>36</sup> Furthermore, miRNA 483-5p inhibits MAPK by acting on it, thereby regulating the mitogen-activated protein kinase (MAPK) cascades. MAPKs participate in signal transduction pathways that control intracellular processes like quick hormonal reactions and substantial changes in an organism's

developmental trajectory<sup>37</sup>. In addition to inhibiting SOCS3 expression, miR 483-5p causes IGF 2 mRNA expression to decline rather than rise<sup>38</sup>. The miR 483-5p target also includes IGF-2.

## 5. DISCUSSION

Preeclampsia, a multisystemic hypertension disorder that affects pregnant women can only be identified 20 weeks after fertilization. The main symptoms are HELLP syndrome, proteinuria, edema, and de novo hypertension. Early diagnosis markers must be developed to assist in selecting the appropriate course of action. Once the placental sickness has advanced, healing is challenging to attain. Therefore, creating early diagnostic markers and implementing early intervention in preeclampsia patients is essential. The placenta has elevated levels of microRNA expression for most mammalian pregnancies.<sup>39</sup> Placental miRNAs control the placenta's development and dynamic environment. For the first time, Pineles et al. looked into the connection between preeclampsia and microRNA. In his study, he talked about two microRNAs (miRNA182 and miRNA210) related to aberrant immune response<sup>40</sup>. Subsequently, Luo et al. discovered that during pregnancy, placenta-specific miRNAs are continually and extracellularly released from chorionic villous trophoblasts into the maternal circulation, where they may target maternal tissues (such as the maternal endothelium).<sup>41</sup> Using computational techniques, the human genome has about 2000 new microRNAs revealed.<sup>42</sup> Nikuei P et al. researched MicroRNA 210's part in immunological dysregulation. In addition, <sup>43</sup> Hromadnikova et al. investigated the efficacy of micro RNAs as a PE44 biomarker. MiRNAs that can be employed as biomarkers in the early detection of PE have been discovered in various research on PE-related miRNAs. m iRNA 210, miRNA 155, miRNA 26, and miRNAs in the 14th chromosomal cluster are a few examples. Also expressed in the current study were these miRNAs. The following conclusions were reached because of the current study's focus on new targets other than the known miRNAs. In the present study, miRNA has-miR-483-5p was overexpressed in preeclampsia patients' placentas. This miRNA was found to

play a role in the negative regulation of cell proliferation and invasion when its functions were examined, suggesting that in preeclampsia, the level of this microRNA was increased compared to normal pregnancy. Genes are responsible for properly developing the placenta, and embryos are targeted by miRNA 483-5p. MiRNA483-5p targets the gene GFRA4 (GDNF family receptor alpha-4) Glial cell line-derived neurotrophic factor (GDNF), which was first discovered to be a significant survival factor for these neurons in animal models of Parkinson's disease, has been shown to protect midbrain dopaminergic neurons. According to some estimates, GDNF is 100 times more effective than neurotrophins at preserving spinal motor neurons. A signaling protein generated from mesenchyme, GDNF also encourages ureteric branching during kidney development. GFRA4 is the conduit through which GDNF acts<sup>45</sup>. The GDNF ligand family's ability to signal and function enables these elements to regulate various activities, from synaptic function and adult regenerative responses to neuronal survival, axon guidance, and synapse formation in the developing nervous system. The human lung expresses GDNF as well<sup>46</sup>. SMAD2 signaling is regulated by miRNA 483-5p. The formation of the three germ layers in the epiblast depends heavily on TGF signaling mediated by Smad2.<sup>47</sup> Smad2/3 signaling mediates its effects by acting on the bone morphogenetic protein (BMP) signaling pathways, which are crucial for embryonic development and cellular homeostasis<sup>48</sup>. According to reports, SMAD2/3 participates actively in endoderm development by various action mechanisms during the differentiation of hESCs into endoderm.<sup>49</sup> SOCS3 and IGF 2 are negatively affected by miRNA 483-5p. It was determined that SOCS3 is a factor that affects leptin-resistant obesity<sup>50</sup>. Mammals have 8 SOCS proteins: SOCS1–7 and a protein with the alternative name Cytokine-inducible SH2-containing protein (CISH). Whereas SOCS4-7 is more frequently implicated in regulating receptor tyrosine kinase (RTK) signaling, SOCS1-3 and CISH are primarily linked to the control of cytokine receptor signaling. In light of this, we propose that the intronic miR-483-5p functions as a crucial player in the pathway involving Igf2-based control of lipid metabolism through Socs3. In many infectious disorders, the SOCS 3 protein plays a role in downregulating inflammatory indicators such as interleukin 6 and IL6/STAT 3 signaling.<sup>51</sup> BCL-2 is another target of miRNA 483-5p. The pro-apoptotic protein BCL-2 is located on the outer membrane of mitochondria, which profoundly impacts cellular viability by preventing it from executing its role. The pro-apoptotic BCL-2 family proteins, including Bax and Bak, often influence the

## 10. ABBREVIATIONS

ACOG -American College of Obstetricians and Gynecologists  
 ACTH -Adrenocorticotropic hormone  
 ADAM -a disintegrin and metalloproteinase  
 ADMA -Asymmetric dimethylarginine  
 BAX - Bcl-2-associated X protein  
 BAK - Bcl-2 homologous antagonist/killer  
 BCL-2 -B cell Lymphoma -2  
 BH3 - The Bcl-2 homology domain 3.  
 BMP - Bone morphogenetic proteins  
 CISH - Cytokine Inducible SH2 Containing Protein  
 CRH - Corticotropin-releasing hormone  
 CTB – Cytotrophoblast  
 EOPE -Early Onset Preeclampsia  
 EVT -Extracellular Vescicles  
 GDNF - Glial cell line-derived neurotrophic factor  
 GFRA4- GDNF receptor alpha-3 GDNFR-alpha-3

mitochondrial membrane to promote permeabilization and release cytochrome-c and ROS, essential signals in the apoptosis cascade. BCL-2 and its related protein BCL-XI impede the functions of BH3-only proteins, activating this pro-apoptotic proteins<sup>52</sup>. The present findings may be useful in developing early diagnosis markers and treatment targets for preeclampsia. MiR-483-5p has been shown to contribute to several malignancies, including oral and tongue squamous cell carcinoma.<sup>53</sup> Due to its impact on cellular biological processes such as cell division, migration, and invasion<sup>54</sup>. There are no reports yet, on miR-483-5p's contribution to the pathogenesis of preeclampsia. Consequently, this study aimed to identify the functions of miR-483-5p in preeclampsia and determine whether or not it may be employed as a biomarker for the early diagnosis of the condition.<sup>55</sup> The signal transduction pathways involved in cell growth, proliferation, and migration are mostly impacted by miRNA 483-5p.

## 6. CONCLUSION

The goal of the current study was to use next-generation techniques to detect the difference in microRNA expression profiles in the placenta between preeclampsia patients and women with normal pregnancies. The present study identified that microRNA 483-5p could be used as a biomarker for the early detection of preeclampsia. The limitation of the study is that the study didn't analyze other microRNAs which could have been used as a biomarker due to economic reasons. However, further research with larger samples and including other miRNAs strengthens this hypothesis.

## 7. AUTHORS CONTRIBUTION STATEMENT

Vinaya Vijayan -Done the sample collection, and analysis of results, and authored the final version of the manuscript. Dr. R. Kannan helped in the analysis part and discussion. Dr. Ram Reddy helped with statistical analysis.

## 8. 10. ACKNOWLEDGEMENT

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## 9. CONFLICT OF INTEREST

Conflict of interest declared none.

HcG -Human Chorionic Gonadotropin  
 HELLP - hemolysis, elevated liver enzymes, and low platelet count.  
 ICAM - intercellular adhesion molecule  
 IUGR - Intrauterine growth restriction  
 IL3 -interleukin 3  
 JAK-STAT - Janus kinase/signal transducers and activators of transcription  
 LOPE -Late Onset Preeclampsia  
 MAPK -Mitogen-Activated Protein Kinase  
 MCP -Monocyte Chemotactic Protein  
 MVB -Multivesicular Bodies  
 MS -Mean of squares  
 NO -Nitric Oxide  
 PIGF -Placental Growth Factor  
 PPI3 -Placental Protein 13.  
 RIN - RNA Integrity Number  
 SOCS - Suppressor of cytokine signaling  
 Sflt/VEGFR-1 - Soluble fms-like tyrosine kinase-1/Vascular Endothelial Receptor type-1.

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