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Gene expression analysis of *MMP*s in women with preeclampsia using cell-free fetal RNA in maternal plasma

Elham Etesami[a,b], Habib Nikukar[a], Fariba Ramezanali[c], Fahimeh Ghotbizadeh Vahdani[d], Maryam Shahhoseini[b], Raha Favaedi[b], Azadeh Ghaheri[e], Sara Seydabadi[a,b], Mohammadreza Zamanian[b,*]

[a] Department of Stem Cell Biology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[b] Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
[c] Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
[d] Obstetrics and Gynecology Department, Imam Khomeini Medical Complex, Tehran University of Medical Sciences, Tehran, Iran
[e] Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

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ABSTRACT

Objective: Nucleic acids released from the placenta into the mother's blood circulation system provide a valuable source of potential biomarkers for early detection of pregnancy complications such as preeclampsia (PE). PE affects nearly 5–10% of pregnancies worldwide and is a major contributor to the maternal and neonatal mortality and morbidity. It is known that altered placental expression of matrix metalloproteinases (*MMP*s) may cause shallow cytotrophoblastic invasion and ultimately lead to preeclampsia. The present study aimed to evaluate pattern of placental/fetal expression of the MMP family (*MMP-2*, *MMP-9*, *MMP-14*, *MMP-15* and *MMP-26*) in preeclamptic women and compare it to normal pregnancies, using cell free fetal RNA (cff-RNA).
Methods: Blood samples were obtained from 20 pregnant women diagnosed with severe PE (28–32 weeks) and 40 control healthy pregnant women in two groups of either matched gestational age (N = 20) or 14 and 28 weeks pregnancies (each 10). cff-RNA was extracted from plasma, followed by reverse transcription of cff-RNA. Expression of *MMP* genes was measured using quantitative reverse transcription PCR (qRT-PCR).
Results: The expression levels of *MMP-2, MMP-9* and *MMP-15* were significantly increased, while *MMP-14* expression level was significantly reduced and the expression of *MMP-26* showed a relative increase in PE pregnancies compared to the control group. Additionally, increased level of *MMPs* expression was observed by comparing 14 and 28 weeks gestation age in normal pregnancy.
Conclusion: Using cff-RNA, circulatory expression level of *MMP-2, MMP-9, MMP-14* and *MMP-15* were significantly altered in preeclampsia compared to normal pregnancies.

1. Introduction

Preeclampsia (PE) is a multisystem [1] and pregnancy-specific disorder [2] that involves many organs including kidney, liver and brain [3]. PE may cause significant maternal and fetal/neonatal morbidity and mortality [4] and even terminate to eclampsia, coma and death [5]. It is characterized by new-onset of hypertension (systolic blood pressure ⩾140 mmHg and/or diastolic blood pressure ⩾90 mmHg) and proteinuria (⩾300 mg/day) after 20 weeks of gestation [6]. In the absence of proteinuria, diagnosis is made based on the association of blood pressure with evidence of systemic disease (such as thrombocytopenia, elevated levels of liver transaminases, renal insufficiency, pulmonary edema and visual or cerebral disturbances) [7,8].

Etiology and pathogenesis of preeclampsia are not yet completely understood. Since some women with hydatidiform mole also develop preeclampsia in the absence of fetus, it is well accepted that placenta is mainly responsible for development of the disease [9]. Several pathways have been described in PE pathogenesis. It is generally acknowledged that defective trophoblastic invasion into myometrium leads to abnormal uterine spiral artery remodeling. The consequence of this shallow implantation is placental ischemia that conduces to local hypoxia and oxidative stress followed by micro-trauma at the level of placenta. Release of the placental fragments into the maternal blood circulation system will then entail systemic inflammatory reaction and

* Corresponding author.
E-mail address: mrzamanian@royaninstitute.org (M. Zamanian).

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Table 1
Previous studies on relationship between MMPs and preclampsia.

Author	Year	Sample	Source	Gene	Result
Fernandez	1999	Protein	Rat	MMP-2	MMP-2 → Endothelin-1↑ vasoconstriction↑
Isaka	2003	Protein	Cytotro-phoblast	MMP2 MMP9	Having role in trophoblast invasion
Pang	2003	RNA	Placenta	*MMP-10* *MMP-13 MMP-15*	Increase in preeclamptic women
Sun	2008	RNA	Blood	*MMP-8*	Increase in preeclamptic women
Founds	2009	RNA	CVS	*MMP-12*	Decrease in preeclamptic women
Mayor	2010	RNA	Placenta	MMP-1	Increase in preeclamptic women
Galewska	2010	Protein	UCB	MMP-26 MMP-7	MMP-26 ↑ MMP-7
Rajakumar	2011	RNA	MBC	*MMP-9*	Increase in preeclamptic women

derivation of free radicals which will eventually terminate in vascular endothelial damage and complications such as hypertension, proteinuria and other systemic responses [10–12]. Currently, the only definitive treatment for PE is delivery of the placenta and pregnancy products [13].

Thus far, several methods -such as medical history, maternal biophysical/biochemical markers (uterine artery doppler ultrasound scan, serum PAPP-A, PlGF and etc.)- are used for screening of PE pregnancies. Combination of these methods show further clinical advantages, despite their limitations due to the undesired sensitivity [14] requirement of larger sample sizes [15–17]. Development of some methods, including Chorionic villus sampling (CVS) test and amniocentesis, provide direct access to the fetal cells for genetic analysis, culminating in early detection and reduction of false positive rate, while their invasive nature has markedly limited their potential applications [18–20]. In this regard, using non-invasive early screening or diagnostic methods seem necessary, with the aim of monitoring high-risk pregnant women and directing available preventive and treatment options in order to reduce maternal and fetal morbidity and mortality.

Discovery of cell-free fetal DNA (cff-DNA) by Lo *et al.* (1997) followed by cell-free fetal mRNA (cff-mRNA) by Poon *et al.* (2014) in maternal blood provided a new possibility in non-invasive diagnosis [21,22]. It has been hypothesized that trophoblastic apoptosis leads to circulating majority of the cff-nucleic acids in maternal blood. Currently non-invasive prenatal test (NIPT) is run as a routine technique to determine fetal sex, RHD genotype and aneuploidy in some diagnostic laboratories [23].

Using cff-RNA has advantages over using cff-DNA for Non-invasive diagnosis:

1. By targeting cff-RNA of the genes that are specifically expressed in placenta, discrimination of fetal and maternal genetic materials will be possible.
2. As a consequence of normal biology, level of the expressed circulating RNA molecules is much higher than that of fetal DNA.
3. Concentration of circulating RNA molecules is variable for different individual genes, unlike the cff-DNAs [24].

Recent studies have focused on the relationship between pregnancy complications and alterations in cell-free fetal nucleic acid contents in maternal blood. For example, modulation in mRNA levels of *CRH* [25–31], *PLAC1* [27,32], *GCM1* [32], *Selectin P* [27], *PAI-1*, *tPA* [33], *VEGF*, *VEGFR1*, *Endoglin* [34], *PSG9* [35], *P21*, *HIF1α* [36] and *PAPPA2* [35] genes in maternal plasma has been reported in conjunction with pregnancy complications.

Degradation of extracellular matrix (ECM) is essential for development, morphogenesis, tissue repair and remodeling [37]. Various types of proteinase are involved in degradation of ECM, among which matrix metalloproteinases (MMPs) are the most important ones [38]. Collectively, 24 different MMPs are capable of degrading ECM components [39,40] and engaged in cellular processes, such as cell proliferation, migration and adhesion. They are also active in fundamental physiological events including tissue remodeling, angiogenesis, bone development, wound healing, uterine and mammary involution [41,42]. Function of MMPs are hindered by tissue inhibitors of metalloproteinases (TIMPs) [43].

MMP-induced downstream pathways affect uteroplacental remodeling and vascular function [44–47]. Previous studies have revealed a relationship between MMPs and increased pregnancy blood pressure, PE or trophoblastic disorders. Fernandez et al. showed that MMP-2 is able to cleave big ET-1, thus generating a vasoconstrictor peptide which can increase blood pressure in rat [48]. Isaka et al. suggested that invasive capacity of trophoblasts may be regulated by the enzyme activity of MMP-2 and MMP-9 [49]. There are also reports on differentially expression of MMPs in preeclamptic pregnancies. Increased expression of *MMP-1* in the placenta of preeclamptic women has been reported in comparison with healthy pregnancies by Mayor-Lynn et al. [50]. Using microarray analysis of peripheral leucocytes, Sun et al. found that *MMP-8* RNA was increased in the preeclamptic patients[51]. Founds et al. also used microarray technique on CVS tissue samples and reported *MMP-12* down-regulation [52]. Pang et al. used this technique on placenta tissue and reported an up-regulation in the *MMP-10, MMP-13* and *MMP-15* [53]. Galewska et al. reported increased level of *MMP-26* in plasma taken from umbilical cord blood of PE pregnancies [54]. Rajakumar et al. observed an increased expression level of *MMP-9* in blood mononuclear cells of PE patient, compared to normal pregnancies [55]. All of the mentioned studies are listed in Tables 1 and 2.

Taking all together, role of *MMPs* on evolution/progression of preeclampsia is undeniable. In the present study, we aimed to determine pattern of *MMP* expressions in preeclamptic pregnancies and compare it to normal pregnancies obtained from healthy mothers, using cff-mRNA, hoping for introduction of new molecular biomarkers being able to non-invasively help early detection of PE pregnancy related complication.

2. Materials and methods

2.1. Study groups

The enrolled population in our study included 20 women at 28–32 weeks of gestational age pregnancy with severe preeclampsia (as case group) and 40 healthy pregnant women (as controls) divided into the following subgroups: (1) gestational age matched controls (N = 20); (2) low risk normal pregnancies at 14 (N = 10) or 28 weeks (N = 10) of gestation.

Severe preeclampsia was defined as blood pressure (BP) of at least 160 mmHg (systolic) and 110 mmHg (diastolic), with proteinuria ≥ 2 on dipstick measured twice with six hours interphase. Patients with chronic high blood pressure before 20 weeks of gestation age (GA), history of kidney disease and gestational diabetes were excluded from the study (Table 3).

Table 2
Previous studies on changes in the concentration of MMPs proteins in PE pregnancies and controls. AF: amniotic fluid, UCB: Umbilical cord blood, CVS: chorionic villous sampling, →↑: increase, ↓:decrease, S: significant, inS insignificant.

Protein	Authors	Year	Source	Study population	↑/↓	S/inS
MMP-2	Hisao Narumiya et al. (2)	2001	plasma	12 PE, 12 Normal pregnancies	↑	S
	JE Myers et al. (3)	2005	plasma	12 PE, 12 Normal pregnancies (22Ws)	↑	S
	Michal Lavee et al. (4)	2009	AF	133 pregnancies (PE + Normal)	↑	S
	Ana C.T. Palei et al. (5)	2012	plasma	130 PE, 130 Normal pregnancies	↑	S
	Eleuterio et al. (6)	2015	plasma	59 PE, 46 Normal pregnancies	↑	S
MMP-9	Galewska et al. (7)	2008	UCB	10 PE, 10 Normal pregnancies	↑	S
	Poon et al. (8)	2009	Serum	128 PE, 296 Normal pregnancies	↑	S
	Palei et al. (9)	2012	Plasma	214 PE, 214 Normal pregnancies	↑	inS
MMP-14	Sitras et al. (10)	2009	Placenta	21 PE, 50 Normal pregnancies	↑	S
MMP-15	Kaitu et al. (11)	2012	CVS	8 PE, 8 Normal pregnancies	↑	S
MMP-26	Galweska et al. (12)	2010	UCB	10 PE, 10 Normal pregnancies	↑	S

2.2. Plasma collection

The study was approved by the Royan Research Institute Ethics Committee, and a written consent from all participants was received. The samples were collected at Imam Khomeini Hospital (Tehran, Iran). 10 ml peripheral whole blood was collected into EDTA-Vacutainer tubes. Immediately after blood collection, plasma was separated from the cellular fraction in two stages. First, the tubes were centrifuged at 1600 × g for 10 min at 4 °C. The supernatant was next transferred into 1.5 ml Eppendorf tube. Separated plasma was re-centrifuged at 16000 × g for 10 min at 4 °C. Then supernatants were carefully transferred into 2 ml cryovials. The plasma samples were snap-frozen and stored at −70 °C until cell free RNA extraction.

2.3. Extraction of cell free fetal RNA and cDNA synthesis

Plasma from each sample was melted and centrifuged at 16000 × g for 5 min at 4 °C. The supernatant was isolated carefully and used for cell-free nucleic acid extraction using QIAamp Circulating Nucleic Acid Kit (QIAGEN, Canada) according to the manufacturer's instruction. In brief, 3 ml of each centrifuged plasma sample was lysed (ACL buffer), followed by binding of fragmented DNA and RNA to a silica membrane (ACB buffer). After passing the sample lysate through the silica membrane (QIAamp Mini column) using a vacuum pump, the bounded nucleic acids were washed by ACW1 and ACW2 buffers, followed by adding alcohol. Finally washed nucleic acids were eluted using elution buffer (AVE) for the future PCR performances. Concentration and purity of the extracted RNA was measured using a Nanodrop2000 spectrophotometer (Thermofisher, USA). Absorbance ratios of A_{260}/A_{280} and A_{260}/A_{230} were determined as indicators of RNA yield purity. Cell-free nucleic acids were stored at −80 °C for further experiments. Considering that cell free RNA maternal fractions are too unstable and degraded very rapidly inside the blood circulation system, the extracted cell free RNA is mostly considered to be composed of fetal orgin.

2.4. cDNA synthesis

Extracted cell-free RNA was recruited in RT reactions for cDNA synthesis. Reactions of 20 µl total volume were prepared using Superscript VILO cDNA Synthesis Kit (Invitrogen, USA) according to the kit protocol provided by the manufacturer.

2.5. Primer design

RNA specific primers for human *18s rRNA* (as internal control), *MMP-2*, *MMP-9*, *MMP-14*, *MMP-15*, *MMP-26* were designed (Table 4) by PerlPrimer version 1.1.21. Primer specificity was investigated by NCBI BLAST.

2.6. Real-Time PCR

Expression level of *18s rRNA*, *MMP-2*, *MMP-9*, *MMP-14*, *MMP-15*, *MMP-26* were assessed using SYBR Green qPCR Master Mix (Applied Biosystems, USA) and StepOne Plus Real-Time PCR System (Applied Biosystems, USA). Running conditions were as follows: primary denaturation at 95 °C for 4 min followed by cycling stage at 95 °C for 10 s and 60 °C for 1 min (for 45 cycles) and then melt curve analysis was started at 95 °C for 15 s (1cycle). Specificity of PCR products were checked by running on an agarose gel. Samples were run in triplicate and data were normalized using *18s rRNA*, while it was calibrated against the average ΔCt of control samples. The results were reported as fold change relative to controls. No template control (NTC) was used in all runs. Finally, the relative expression of target genes was calculated by the comparative cycle threshold method (ΔΔCt).

2.7. Statistical analysis

Data were analyzed using statistical package for social science (SPSS) software version 16. Kolmogorov–Smirnov test was used to review the normal distribution (adopted when p-value > 0.05). Student's *t*-test was used to check significance of parametric variables. Data were shown as the mean ± standard deviation (mean ± SD) and $p < 0.05$ was considered statistically significant.

Table 3
Demographic and biochemical variables in normal pregnant and preeclamptic women.

Author	Normal Pregnant Women (in 14 weeks)	Normal Pregnant Women (in 28 weeks)	Normal Pregnant Women (match controls)	Severe Preeclamptic Women
n	10	10	20	20
Age (y)	34 (28–41)	32 (27–41)	31 (22–40)	32 (23–41)
SBP (mmHg)	11 (10–12)	11 (10–12)	11 (10–12)	17 (15–19)
DBP (mmHg)	8 (7–9)	8 (7–9)	8 (7–9)	11 (10–12)
Proteinuria	–	–	–	+3 (+2 - +4)
Gestational Age(w)	14 (13–14)	28 (28–29)	30 (28–32)	30 (28–32)

Table 4
List of designed primers for selected *MMP* genes.

Gene	Primer sequences (5′ → 3′)	Product size (bp)
18srRNA	F: 5′ GTAACCCGTTGAACCCCATT 3′ R: 5′ CCATCCAATCGGTAGTAGCG 3′	151
MMP-2	F: 5′ GGAAAGATGTGGTGTGCGA 3′ R: 5′ CTTGGTGTAGGTGTAAATGGGT 3′	176
MMP-9	F: 5′ CAATCTCACCGACAGGCA 3′ R: 5′ ACTCTCCACGCATCTCTG 3′	80
MMP-14	F: 5′ CTGTGACGGGAACTTTGAC 3′ R: 5′ GTAGGCAGTGTTGATGGAC 3′	162
MMP-15	F: 5′ ACGAGTGAAAGCCAACCT 3′ R: 5′ TACCAGCCCAACTTCTCC 3′	116
MMP-26	F: 5′ GGATGGGACTTTGTTGAGG 3′ R: 5′ GCTGTGTTTGTGTCTCCTG 3′	87

Table 5
List of selected MMP genes(1).

Official Symbol	Official full name	Protein name	functions
MMP-2	matrix metallopeptidase-2	collagenase type IV-A	Angiogenesis blood vessel maturation embryo implantation response to hypoxia
MMP-9	matrix metallopeptidase-9	type V collagenase macrophage gelatinase	collagen catabolic process embryo implantation negative regulation of apoptotic process
MMP-14	matrix metallopeptidase-14	membrane-type-1 MMP	angiogenesis collagen catabolic process
MMP-15	matrix metallopeptidase-15	membrane-type-2 MMP	proteolysis
MMP-26	matrix metallopeptidase-26	Matrilysin 2	Is not known Completely

3. Results

Using Google Scholar, National Centre for Biotechnology Information (NCBI) and Gene data banks (A portal to gene-specific content based on NCBI's RefSeq project), we identified some of *MMP* genes correlated with PE pathophysiology. Following an in depth review of literature, five candidate genes (Table 5) were selected for evaluation.

We measured expression level of the selected MMPs in the maternal plasma cff-RNA with preeclamptic and control pregnancies. Findings showed significant increase in *MMP-2*, *MMP-9* and *MMP-15* expression levels of the preeclamptic group compared to the matched controls. In contrast, *MMP14* showed a significant decrease in the case group compared to the matched control. For *MMP-26*, we could detect a relative increase in its expression level by comparing patients to the normal matched pregnancies ($P < 0.05$) (Fig. 1).

We also tried to determine expression levels of the *MMPs* in the early stage of normal pregnancies (14 weeks of GA) and compare it with normal pregnancies of 28 weeks of GA. All *MMPs* showed detectable expression in pregnant women at 14 weeks of GA (Fig. 2), while their expression levels were relatively increased at 28 weeks of GA.

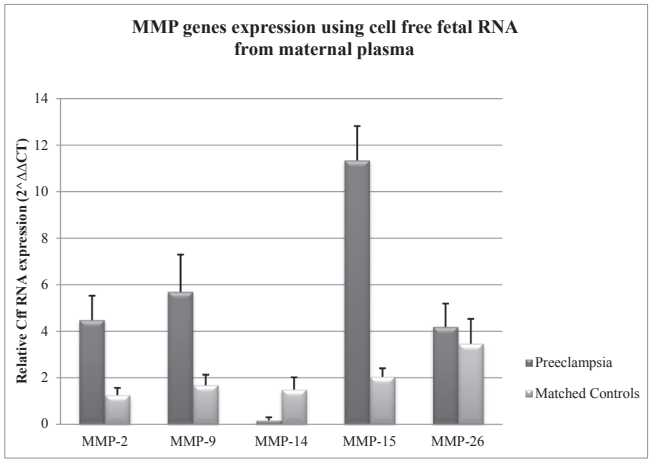

Fig. 1. Plot of Real-time PCR analysis of MMP-2, MMP-9, MMP-14, MMP-15 and MMP-26 gene expression in maternal plasma of preeclamptic (PE) pregnancies versus their matched control group using cell free fetal RNA. *MMP-2*, *MMP-9* and *MMP-15* showed significant increase in PE pregnancies compared to matched control. *MMP-14* showed a significant decrease and *MMP-26* showed a relative increase. (*p-value < 0.05).

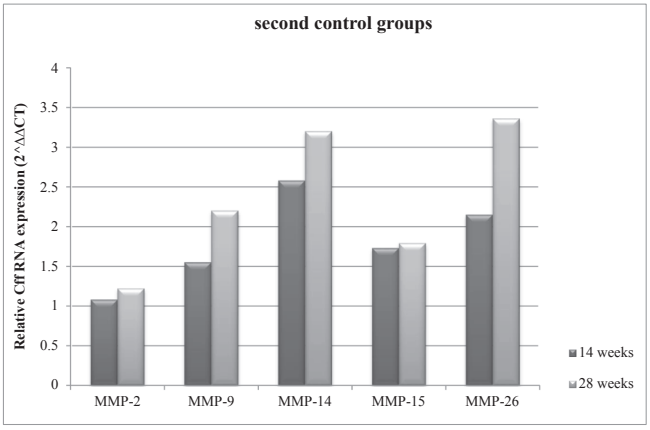

Fig. 2. Plot of Real-time PCR analysis of MMP-2, MMP-9, MMP-14, MMP-15 and MMP-26 gene expression in maternal plasma of 14 weeks healthy pregnancies in comparison with 28 weeks normal pregnancies using cell free fetal RNA. Enhanced expression in 28 weeks compared to 14 weeks (along with the increase in the placental mass) indicates placenta as the source of detected cell free RNAs.

4. Discussion

In the process of endometrial invasion, trophoblastic cells need to pass through ECM. This phenomenon is possibly controlled by establishment of a proper balance between *MMPs* and *TIMPs*. Disturbances affecting *MMPs/TIMPs* balance can lead to PE evolution and progression [56]. Considering the very close relationship between *MMPs/TIMPs* function and PE pathophysiology, we measured expression level of cff-RNA *MMPs* in the plasma of PE pregnancies and compared it with healthy matched controls, aiming to evaluate the possibility of using them as early detection biomarkers in PE. Our results showed significant increase in the expression levels of *MMP-2*, *MMP-9* and *MMP-15* and a significant decrease in the expression level of *MMP-14* in preeclamptic pregnancies compared to the matched controls.

Several studies have previously worked on the expression of *MMP* genes at the protein level (Table 4). But, in our knowledge, this is the first study which is investigating expression level of *MMPs* in maternal blood using cff-RNA.

MMP-2 (gelatinase A (and MMP-9 (gelatinase B) degrade collagen

IV, collagen V, laminin, elastin and many other ECM components as well as soluble proteins. These two proteins are expressed by trophoblast cells and they are considered as the key enzymes in the invasion process [57,58]. It is known that MMP-2 and MMP-9 have a major role during implantation and invasion processes [59].

As mentioned, trophoblast superficial invasion causes an inappropriate rearrangement of spiral vessels and ultimately leads to hypoxia in the placenta [10]. Recent studies in preeclamptic women has revealed an increase in the circulating concentration of angiotensin II type I receptor agonistic autoantibody (AT1-AA) as the result of oxygen deficiency [60]. Anoxia and increased AT1-AA can increase the amount of Hif-1α (hypoxia-inducible factor-1α) mRNA and protein.

Vascular endothelial growth factor *(VEGF)* is one of the genes upregulated by HIF-1α and it is the primary cytokine related to angiogenesis [61]. In Müller cells, increase in Hif-1α induces expression of VEGF. Consequently, VEGF promotes MMP-2 expression and activity in neighboring endothelial cells (ECs) [62] and MMP-9 expression in HUVEC cells [63].

Hypoxia also stimulates production of TNFα. There are reports indicating increased concentration level of blood circulatory in preeclamptic women [64–70]. Cohen *et al.* reported that TNF-α activates two different pathways in trophoblastic cells which in turn will lead to enhancement of the *MMP-9* expression: (1) Erk1/2 pathway which in turn initiates NF-κB activation and (2) SAPK/JNK pathway that activates AP-1 [71]. The promoter region of the *MMP-9* gene contains two AP-1 binding sites. Therefore, increase in AP-1 can foster MMP-9 activity and mRNA production [72].

MT1-MMP (MMP-14) and *MT2-MMP (MMP-15)* are expressed in human placenta during the first trimester of pregnancy [73–75]. They act as activators of some of the secreted MMPs proenzymes, especially for MMP-2 [76,77]. It is shown that MMP-14 could be one of the key enzymes in the process of trophoblast invasion, acting alone or as a cell-surface activator of other proteinases [75]. MMP-14 null mice display impaired basic fibroblast growth factor–mediated corneal angiogenesis [78], and tissues from the same mice fail to show angiogenesis in a type I collagen ex vivo model, supporting the potential role of MMP-14 in angiogenesis [79]. A Research Reported that selective inhibition of MMP-14 inhibits tumor angiogenesis in part, through inhibition of VEGF-driven cell invasion and proMMP-2 activation [80]. Analysis of tumor breast cancer tissue sections revealed that MMP-14 blockade limited tumor neoangiogenesis [81]. As MMP-14 is involved in the process of cell invasion and angiogenesis, its down-regulation decreases trophoblastic cell migration, invasion and neoangiogenesis.

In response to hypoxia, the placenta secretes soluble substances such as anti-angiogenic protein soluble fms-like tyrosine kinase-1 (sFlt-1), inflammatory cytokines and AT1-AA into the maternal blood circulation, causing in turn symptoms of the disease. These factors are capable of inducing hypertension through the production of endothelin-1 (ET-1), that is a potent vasoconstrictor [82]. Several studies have pointed to the increased ET-1 level in plasma [83,84], Serum [85] and placenta [86] of PE pregnancies compared to normal pregnancies. ET-1 in pathologic concentrations down-regulates MMP-14 expression. [87].

TNF-α, which is elevated in preeclamptic women [64–69], increases ET-1 expression and vice versa. This would exacerbate the decline in MMP-14 expression mediated with decreased endothelin-1 [88].

Furthermore, it has been reported that MMP-2 increases the amount of endothelin [48]. By increasing MMP-2 in PE patients, endothelin levels will also be increased and thereby the expression level of MMP-14 will be decreased.

As noted, TNF-α shows an increase in PE cases [64–70]. Treating ACH-3P (a hybrid cell line) with TNF-α in HLA-G positive cases (which are similar to many EVT cells for many markers) lead to increased expression level of MMP-15 [89]. In three cancer cell lines, induction of hypoxia condition led to a significant increase in MMP-15 mRNA and protein expression levels. Further analyses indicated that there were two hypoxia-responsive elements (HREs) in the *MMP-15* promoter, among which HRE1 is essential for transcription activation in oxygen deficient conditions. HIF-1α (that is increased in anoxia and hypoxia) specifically and directly binds to the promoter of *MMP-15* and improves expression level of the gene in hypoxic conditions [90].

MMP-26 (matrilysin-2) is an epithelial enzyme. It is suggested that MMP-26 may play a role in tissue-remodeling processes associated with placentation [91]. MMP-26 can proteolyze multiple components of the ECM, including fibronectin, type IV collagen, vitronectin, gelatins and fibrinogen. MMP-26 is also indirectly involved in decomposition of the ECM molecules by activating MMP-9 [92,93]. *MMP-26* has recently been discovered and it is less studied among the other *MMP* family members [94]. The only report to date, emphasizes on the increased expression of *MMP-26* by the GnRH I and GnRH II hormones in trophoblastic cells [95].

In the previous studies, there was no evidence of increased expression level of GnRH I and GnRH II in preeclamptic patients compared to the normal pregnancies. So, finding mechanism of the increased expression level of *MMP-26* in PE requires further research and navigate potential effect of the other factors involved in the expression of *MMP-26* as well as PE pathogenesis.

Here, we have proposed potential mechanism of MMPs contribution to the PE pathophysiology in a summarized schematic diagram (Fig. 3).

In the second control group, composed of healthy pregnancies either in 14 or 18 weeks of GA, we clearly and relatively detected increasing levels of *MMPs*. In fact, we demonstrated that *MMPs* are transcriptionally expressed in maternal blood from the early pregnancy (14 weeks of GA) and meanwhile this expression level will be increased up to 28 weeks. These are prerequisites for assigning a potential biomarker. In addition, our finding might confirm placental origin of the measured cff-RNAs in plasma. In fact, with raising GA which is in parallel to the placental mass growth, relative expression level of *MMPs* has also been increased.

5. Conclusion

Based on the present data, we report significant alterations in circulating blood cff-RNA expression level of MMPs in preeclampsia. Our findings may help better understand molecular mechanisms involved in disease evolution/progression. Considering the non-invasive nature of cff-RNA application, this result may also confirm the possibility of using *MMPs* as potential biomarkers in early detection of preeclampsia. Future studies are required for further confirming this possibility, in addition to evaluate if MMPs/TIMPs can be used as potential pharmaceutical targets in preeclampsia prevention/treatment. This may become possible through modulation of gene expression to reduce the

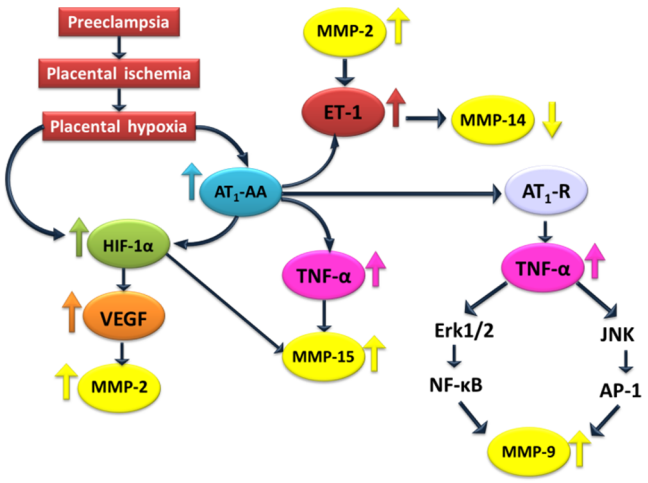

Fig. 3. Proposed pathways for the mechanisms involved in *MMPs* alterations in maternal plasma of preeclamptic pregnancies.

complications and symptoms of the disease. In addition, the scrutiny on cff-RNA also helps identify the source of variations in gene expression. Ultimately, final confirmation of the proposed results and their possible applications require further prospective studies with larger sample sizes.

5.1. What is already known about this topic?

Preeclampsia may cause significant maternal and fetal morbidity and mortality. Etiology and pathogenesis of preeclampsia are not completely understood until now, but it is well accepted that the placenta is mainly responsible for development of the disease. Currently, the only definitive treatment for PE is delivery of the placenta and pregnancy Termination.

Nowadays, several methods such as the use of medical history, maternal biophysical/biochemical markers are used for screening of PE pregnancies. These methods lack desired clinical advantages. On the other hand, techniques such as CVS and amniocentesis which provides direct access to fetal cells for genetic analysis have invasive nature.

Discovery of cff-DNA and cff-mRNA in maternal blood provided a new hope in non-invasive diagnosis of PE.

Recent studies have focused on the relationship between pregnancy complications and alterations in cell-free fetal nucleic acids content in maternal blood. For example, modulation in mRNA levels of *CRH*, *PLAC1*, *GCM1*, *Selectin P*, *PAI-1*, *tPA*, *VEGF*, *VEGFR1*, *Endoglin*, *PSG9*, *P21*, *HIF1α* and *PAPPA2* genes in maternal plasma has been reported in conjunction with pregnancy complications.

Previous studies have revealed a relationship between MMPs and increased pregnancy blood pressure, PE or trophoblastic disorders.

5.2. What does this study add?

Using cff-RNA, circulatory expression of *MMP-2, MMP-9, MMP-14* and *MMP-15* were significantly altered in preeclamptic women compared to normal pregnancies. The stable level of *MMP* expressions during pregnancy may introduce them as potential biomarkers for non-invasive screening of preeclampsia.

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Appendix A. Supplementary data

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