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Original article

Preliminary study on the role of human defensins, interleukins and PCSK9 in early and late preeclampsia

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ABSTRACT

The lack of reliable methods for preeclampsia (PE) early diagnosis limits the opportunities for timely prevention, diagnosis and treatment. This study aims to identify the alterations of biochemical parameters and the immune system activity to build a panel of markers that can support preeclampsia diagnosis. For this study, we recruited 30 pregnant women: 10 healthy pregnant women (CTR); 10 pregnant women with early preeclampsia (EP); 10 pregnant women with late preeclampsia (LP). We evaluated lipid profile and, by gene expression, we assessed PCSK9, IL-2, IL-6, IL-8, IL-10, TNF-α and TGF-β. Moreover, we evaluated both the serum and gene levels of the defensins HBD-1, HBD-2, HBD-4 and HNP-1. Our results showed an increase in gene expression levels of IL-6 and IL-8 in EP compared to LP (IL-6: median 11.7 vs 3.3, $p = 0.005$; IL-8: median 634.1 vs 214.1, $p = 0.013$) and to CTR (IL-6: median 11.7 vs 0.5, $p < 0.001$; IL-8: median 634.1 vs 225.6, $p = 0.012$), highlighting a massive activation of immune system in case of more severe preeclampsia. Furthermore, higher serum levels of HBD1 in LP compared to CTR (median: 278.8 vs 67.8, $p = 0.005$) and to EP (median: 278.8 vs 68.6, $p = 0.001$) might indicate that the same immune system puts in action protective actions to prevent adverse outcome in these cases. Finally, gene expression levels of PCSK9 decreased significantly in women with EP compared to controls and to LP (median: 0.2 vs 0.9, $p = 0.010$; median: 0.2 vs 1.2, $p = 0.012$), causing a decrease in circulating LDL-c necessary for the synthesis of placental hormones.

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1. Introduction

Preeclampsia (PE) is a severe complication of pregnancy, associated with a high rate of maternal and perinatal morbidity and mortality. Even if it is quite common, affecting up to 5 % of pregnancies, its pathophysiology remains unclear [1]. Clinically PE usually develops after 20 weeks and it is characterized by gestational hypertension, proteinuria and/ or signs of organ damage [2]. This condition has been classified into "early PE" (EP) and "late PE" (LP) according to the time of development. The first one develops < 34 weeks of gestation and it is generally more severe and often associated with placental insufficiency, fetal growth restriction, and adverse maternal and neonatal outcome; the latter generally develops ≥ 34 weeks of gestation, and it is associated with a normally grown fetus delivered at or near term.

PE has been recognized as a cardiovascular risk factor. During the first trimester of pregnancy, maternal total cholesterol levels increase by 30 %−50 % due to increased cholesterol synthesis in the liver, for meeting foetal cholesterol demands during organogenesis. Some studies have found associations between low maternal serum cholesterol and adverse pregnancy outcomes, including microcephaly and growth retardation [3–5].

Proprotein convertase subtilisin/kexin 9 (PCSK9) is a serine protease synthesized by the liver and intestine involved in lipid metabolism, degrading LDL-receptor (LDL-R) and high levels of PCSK9 lead an increased risk of cardiovascular disease (ASCVD). The extent which PCSK9 contributes to lipid metabolism during pregnancy is not so clear, however, many pathological conditions such as fetal growth restriction or preeclampsia presented altered levels of PCSK9 [6,7]. On the other hand, a transient increase in Lipoprotein a [Lp(a)] levels has been observed in acute phase condition, such as endothelial damage [8]. Lp (a) transports large amounts of cholesterol to the cells of the vascular wall to promote their regeneration, resulting in an increased risk of atherosclerosis. Moreover, the endothelial damage can cause pathophysiological changes in pre-eclamptic women such as vascular dysfunction, decreased organ perfusion, activation of the coagulation cascade, and increased capillary permeability, leading to some complications such as placental abruption [9,10].

An abnormal immune adaptation to the pregnancy and an increased inflammation might be possible mechanisms underlying the pathophysiology of PE.

Several studies have demonstrated a strong association between intrauterine bacterial or viral infections and pregnancy complications such as abortion, preterm delivery, and PE [11–13]. Natural antimicrobials have been studied in several pregnancy complications including PE [14] and have been proposed as biomarkers of intrauterine infection [15]. The identification of Human Neutrophil Peptide 1 (HNP1) in amniotic fluid is strongly predictive of intrauterine infection while elevated levels of HNP in maternal plasma are a marker of the presence of chorioamnionitis in women with PROM (Premature rupture of membranes) [16]. High concentrations of HNP reflect neutrophil activation in association with labor [17], uterine infection, which seems to play a key role in protecting the foetus [18]. Human Beta Defensin 1 and 3 (HBD1 and HBD3) localized to placental and chorion trophoblast, amnion epithelium and decidua. Human beta-defensin 2 (HBD-2) is expressed in the urinary tract, gastrointestinal tract, respiratory system, skin epithelium and has been recently demonstrated in the amniotic fluid of women with pre-term pregnancy [19]. Human β-defensin 4 (HBD-4) was found in the stomach, testes and uterus [20]. However, elevated expression results in increased antimicrobial protection.

HBD-1 is involved in the first line of defence expressed in genital and respiratory tract epithelial cells [21]. In recent years, numerous studies have found an increase of these peptides not only in case of inflammations but also in case of bacterial or viral infection [22–25].

At physiological conditions, the maternal innate immune system plays a leading role during the entire gestation period [26,27]. Immune cells, such as natural killer cells, dendritic cells and regulatory T lymphocytes located in the decidua, maintain immune tolerance towards spiral artery remodelling and emerging foetal trophoblast [28].

The lack of reliable methods for early diagnosis of PE limits the opportunities for timely prevention, diagnosis and treatment [29]. Current standard care relies heavily on risk assessment and monitoring [30]. The available treatments do not address molecular mechanisms and delivery has been proposed as the only possible treatment.

In this scenario, the aim of our study was to investigate: a) lipid profile; b) gene expression of pro-inflammatory and anti-inflammatory cytokines such as Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interlukin-10 (IL-10), Tumor Necrosis Factor alpha (TNF-α), Transforming Growth Factor beta (TGF-β) and defensins such as HNP-1, HBD-1, HBD-2 and HBD-4; c) serum levels of HNP1, HBD1 and HBD4, in order to identify biomarkers of the immune system of mothers with PE.

2. Materials and methods

2.1. Ethical approval

The study was leaded as stated by the ethical recommendations of Helsinki Declaration of the World Medical Association and was endorsed by the ethics committee (protocol 80/19) of the University of Naples Federico II.

2.2. Study design and study population

This was a prospective observational study. We have chosen a convenience sampling composed by thirty pregnant women, recruited at the Mother and Child Department of University Hospital Federico II and grouped as follows: healthy pregnant women (CTR, n = 10); pregnant women with Early Preeclampsia (EP, n = 10); pregnant women with Late Preeclampsia (LP, n = 10). All patients were advised of the aim and proceedings of the study, and written informed consent was acquired from each participant.

PE was defined according to ISSHP guidelines. We divided patients into EP and LP groups according to the time of development (<34 weeks of gestation in EP, and ≥34 weeks of gestation in LP) [31].

We included only patients undergoing a cesarean section (CS), to avoid bias related to mode of delivery. Patients in active labor were excluded.

2.3. Samples collection

A maternal serum specimen and a placental specimen for each enclosed patient/control were collected the day of the delivery before the beginning of the CS.

Maternal blood was collected utilizing a BD vacutainer (Becton Dickinson, Oxfordshire, UK) blood collection tube with serum separator. After centrifugation for five minutes at 3700 rpm, the sample was instantly frozen at −80 °C up to the time of analysis. Placentas were collected and sampled in the central area of the placental disc, dismissing the maternal decidua and collecting the underlying villi [32]. Samples have been frozen at −80 °C up to the time of the analysis.

2.4. Biochemical determinations

Lipid profile (total cholesterol, low-density lipoprotein, high-density lipoprotein) and C reactive protein (CRP) were evaluated on Architect c16000 (Abbott Diagnostics, Chicago, IL, USA).

2.5. Elisa assay

Lp(a) was estimated in the sera by *ELISA* (Human Lp(a) ELISA (10–1106-01), Mercordia, Uppsala, Sweden, EU) according to the manufacturer's recommendations. HNP1, HBD1 and HBD4 were evaluated in the sera utilizing *ELISA* (Human DEFα1, Human DEFβ1 and

Human DEFβ4 ELISA Kit, Elabscience, Buckingham, UK) according to the manufacturer's recommendations.

All analysis was performed in triplicate to assure the precision of results.

2.6. RNA extraction and cDNA synthesis

Total RNA was obtained from the placenta (one fragment of 50 mg for each sample) by mechanical homogenisation with Ultra Turrax in Trizol Reagent according to the manufacturer's protocol (Life Technologies). The amount of total extracted RNA was calculated by measuring the absorbance at 260 nm and the purity by 260/280 and 260/230 nm ratios by Nanodrop (ND-1000 UV-Vis Spectrophotometer, NanoDrop Technologies). For each sample, 1000 ng of total RNA was retrotranscribed with iScriptTM cDNA synthesis kit (Bio-Rad), according to the manufacturer's instructions.

2.7. Gene expression by real-time qPCR

For real-time qPCR experiments, the data from each cDNA sample were normalized using the human housekeeping gene RLP0 (ribosomal protein lateral stalk subunit P0). The specific primers reported in Table 1 were designed based on the nucleotide sequences downloaded by NCBI database using Primer3WEB v.4.0.0.

Calculations of relative expression levels were performed using the $2^{-\Delta\Delta Ct}$ method. All analysis were performed in triplicate to guarantee the accuracy of results.

2.8. Statistical analysis

Descriptive analysis was carried out using median and interquartile range (IQR). Normality distribution was assessed by the Shapiro-Wilk. Univariate comparisons were investigated between groups (CTR, EP and LP) and explicative variables using Kruskal Wallis test followed by Sign test with Bonferroni's correction for multiple comparisons. A statistical significance was set at the level of ≤ 0.05, unless adjustment for multiple comparisons was needed (in this case the significance threshold was 0.0167 (p/k, assuming k = 3 contrast)). All analyses were performed using Stata software v18 (StataCorp, College Station, USA).

3. Results

3.1. Characteristics of the study population

This study involved 30 pregnant women divided as follows: CTR (N = 10); EP (N = 10); LP (N = 10). The main characteristics of the study population have been described in Table 2.

Table 2
Median and interquartile interval (25th and 75th percentile) values for age, pre-pregnancy weight, pre-partum weight,pre-pregnancy BMI, pre-partum BMI and gestational age at birth in three different group: control group (CTR), early preeclampsia pregnancy (EP) and late preeclampsia pregnancy group (LP); p-value for Kruskal-Wallis test; * *p-value < α/3 for Bonferroni multiple testing correction.

	Total	CTR	EP	LP	p-value
	N = 30	N = 10	N = 10	N = 10	
Age	32.5 (30.0 −35.0)	34.5 (30.0 −38.0)	32.0 (28.0 −35.0)	31.5 (30.0 −33.0)	0.271
Height (m)	1.60 (1.60 −1.70)	* *1.60 (1.60 −1.60) vsLP	* *1.60 (1.60 −1.70) vsLP	1.70 (1.70 −1.70)	< 0.001
Pre-pregnancy weight (kg)	67.5 (60.0 −75.0)	64.5 (55.0 −73.0)	67.0 (64.0 −71.0)	74.0 (63.0 −80.0)	0.248
Pre-partum weight (kg)	77.5 (71.0 −84.0)	74.0 (65.0 −84.0)	75.5 (65.0 −80.0)	83.0 (79.0 −91.0)	0.060
Pre pregnancy BMI (kg/m2)	25.8 (22.9 −27.4)	24.1 (23.0 −26.8)	25.7 (22.1 −27.0)	27.1 (22.7 −28.3)	0.560
Pre-partum BMI (kg/m2)	29.0 (26.6 −31.0)	28.9 (27.7 −30.9)	29.2 (23.5 −31.0)	29.2 (28.3 −31.0)	0.859
Gestational age at birth (i.e days)	270.0 (220.0 −274.0)	* *274.0 (272.0 −278.0)vs EP	* *207.0 (199.0 −220.0)vs LP	270.0 (251.0 −280.0)	< 0.001

3.2. Detection of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), lipoprotein (a) and C reactive protein (CRP) in pregnant women with PE

To evaluate if PE can alter the lipid profile in pregnant women, we analyze main parameters involved in the metabolism of the cholesterol (Table 3). The results show that there are no significant differences in Total Cholesterol, LDL, HDL, Lp(a) and CRP between both the LP and EP groups compared to CTR one.

3.3. Effect of PE on the gene expression of cytokines, defensins and PCSK9

To highlight the activation of the immune system of pregnant women complicated by PE, we estimated the levels of gene expression of some cytokines and AMPs (Table 4). For IL-6 and IL-8, we observed a significant difference between groups ($p < 0.001$ and $p = 0.014$, respectively); in particular, both cytokines increased in EP compared to CTR and LP (Fig. 1 A and 1B) and IL-6 also increased in LP compared to CTR. Regarding IL-2, we can note an up-regulation in EP, compared to controls. Instead, IL-10 is slightly decreased in EP compared with control

Table 1
Primer sequences were designed with Primer3WEB v.4.0.0 and used for qPCR.

Gene	Forward	Reverse	NM
RLP0	5'-TGGCAGCATCTACAACCCTG−3'	5'-GACAAGGCCAGGACTCGTTT−3'	NM_053275.4
TNF-α	5'-CAAGGACAGCAGAGGACCA−3'	5'-CGTCCCGGATCATGCTTTCA−3'	NM_000594.4
TGF-β	5'-GGTGAGGAAACAAGCCCAGA−3'	5'-TGCCTCCCAAAAGTGCTAGG−3'	NM_000660.7
IL−2	5'-AACCTCAACTCCTGCCACAA−3'	5'-GCATCCTGGTGAGTTTGGGA−3'	NM_000586.4
IL−6	5'-CATCCTCGACGGCATCTCAG−3'	5′-TCACCAGGCAAGTCTCCTCA−3	NM_001318095.2
IL−8	5'-AAACCCAGGTGAGAGCTG−3'	5′-TCTGAGATCCCGTCAGAGC−3'	NM_001354840.3
IL−10	5'-TCCATTCCAAGCCTGACCAC−3'	5'-AATCCCTCCGAGACACTGGA−3'	NM_001382624.1
HBD1	5'-TTTTGTCTGAGATGGCCTA−3	5'-GGGCAGGCAGAATAGAGACA−3'	NM_005218.4
HBD2	5'-ATCAGCCATCAGGGTCTTGT−3'	5'-GAGACCACAGGTGCCAATTT−3'	NM_004942.4
HBD4	5'-AGATCTTCCAGTGAGAAGCGA−3'	5'-GACATTTCTTCCGGCAACGG−3'	NM_000008.11
HNP1	5'-CATCCTTGCTGCCATTCTCC−3'	5'-CCTGGTAGATGCAGGTTCCA−3'	NM_004084.4
PCSK9	5'-TGGTGAAGATGAGTGGCGAC−3'	5'-TCCCGGTGGTCACTCTGTAT−3'	NM_174936.4

Table 3
Median and interquartile interval (25th and 75th percentile) values for Total Cholesterol, LDL, HDL, Lpa and CRP in three different group: control group (CTR), early preeclampsia pregnancy (EP) and late preeclampsia pregnancy group (LP); p-value for Kruskal-Wallis test.

	Total	CTR	EP	LP	p-value
	N = 30	N = 10	N = 10	N = 10	
Total Cholesterol (mg/dL)	242.5 (229.0 −262.0)	281.5 (239.0 −330.0)	240.5 (213.0 −251.0)	237.5 (229.0 −250.0)	0.086
LDL (mg/dL)	127.5 (109.0 −143.0)	141.5 (126.0 −203.0)	119.0 (103.0 −130.0)	114.5 (109.0 −135.0)	0.094
HDL (mg/dL)	70.0 (57.0 −76.0)	68.5 (50.0 −78.0)	72.5 (67.0 −75.0)	63.5 (46.0 −77.0)	0.500
Lpa (mg/dL)	2.0 (1.7 −2.5)	2.0 (1.9 −2.8)	2.0 (1.7 −2.3)	2.2 (1.7 −2.5)	0.541
CRP (mg/L)	0.7 (0.4 −1.0)	0.8 (0.5 −1.9)	0.6 (0.3 −0.7)	0.8 (0.4 −3.4)	0.326

and LP, but it is not significant. TNF-α and TGFβ have a similar trend: both are increased in EP and in LP compared with the control group, but without statistical significance.

We also examined the gene expression of HNP-1, HBD-1, HBD-2 and HBD-4. The only one defensin that showed a significant difference in three groups was HBD-1, $p = 0.018$; increasing in EP compared with CTR (Fig. 1 C).

Finally, PCSK9 showed a different trend ($p = 0.010$), with a decrease in EP compared to CTR and an increase in LP compared to EP. Instead, there is no difference between control group and LP (Fig. 1D).

3.4. Estimation of HNP-1,HBD-1 and HBD-4 levels in serum of mothers affected by PE

To detect a possible accumulation of HNP-1, HBD-1 and HBD-4 in the maternal serum of pregnant women with PE we performed an *ELISA* on serum (Table 5). We found a significant increase of HBD-1 between groups ($p = 0.002$): in particular in LP women compared to EP ($p = 0.005$) and CTR ($p = 0.001$) (Fig. 2). HNP-1 shows an increasing trend in LP compared to EP and controls, it is not statistically significant

Table 4
Median and interquartile interval (25th and 75th percentile) values for IL-2, IL-8, IL-6, IL-10, TNFα, TGFβ, HBD1, HBD2, HBD4, HNP1 and PCSK9 in three different group: control group (CTR), early preeclampsia pregnancy group (EP) and late preeclampsia pregnancy group (LP); p-value for Kruskal-Wallis test; * *p-value < α/3 for Bonferroni multiple testing correction.

	Total	CTR	EP	LP	p-value
	N = 30	N = 10	N = 10	N = 10	
IL−2 (ΔΔct)	71.2 (39.4 −100.3)	54.6 (10.0 −81.8)	245.2 (81.3 −589.3)	61.0 (50.9 −90.9)	0.030
IL−8 (ΔΔct)	243.9 (162.5 −400.9)	* *225.6 (55.9 −294.8)vs EP	* *634.1 (256.2 −703.0) vs LP	214.5 (90.5 −325.6)	0.014
IL−6 (ΔΔct)	2.5 (0.5 −7.0)	* *0.5 (0.3 −0.6)vs EP; vs LP	* *11.7 (6.3 −15.5) vs LP	3.3 (1.4 −6.2)	< 0.001
IL−10 (ΔΔct)	597.3 (306.7 −823.7)	686.4 (316.2 −918.8)	442.1 (301.3 −763.6)	641.1 (486.0 −742.3)	0.771
TNFα (ΔΔct)	377.2 (149.3 −500.4)	255.8 (31.4 −747.9)	478.2 (391.5 −510.7)	313.4 (69.8 −397.0)	0.278
TGFβ (ΔΔct)	425.9 (217.6 −648.5)	278.1 (47.1 −551.0)	472.6 (217.6 −695.1)	460.6 (378.2 −985.5)	0.155
HBD1 (ΔΔct)	0.2 (0.2 −1.2)	* *0.1 (0.0 −0.3)vs EP	0.5 (0.2 −2.0)	0.2 (0.2 −1.5)	0.018
HBD2 (ΔΔct)	0.0 (0.0 −0.1)	0.0 (0.0 −0.1)	0.0 (0.0 −0.1)	0.0 (0.0 −0.1)	0.996
HBD4 (ΔΔct)	14.2 (10.4 −22.4)	12.2 (5.4 −17.7)	15.5 (14.1 −23.1)	11.9 (10.6 −17.8)	0.308
HNP1 (ΔΔct)	4.6 (2.7 −15.2)	3.2 (2.2 −27.4)	6.5 (3.9 −9.0)	3.7 (3.4 −5.5)	0.537
PCSK9 (ΔΔct)	0.5 (0.3 −1.9)	* *0.9 (0.3 −3.5)vs EP	* *0.2 (0.1 −0.5) vs LP	1.2 (0.3 −2.3)	0.010

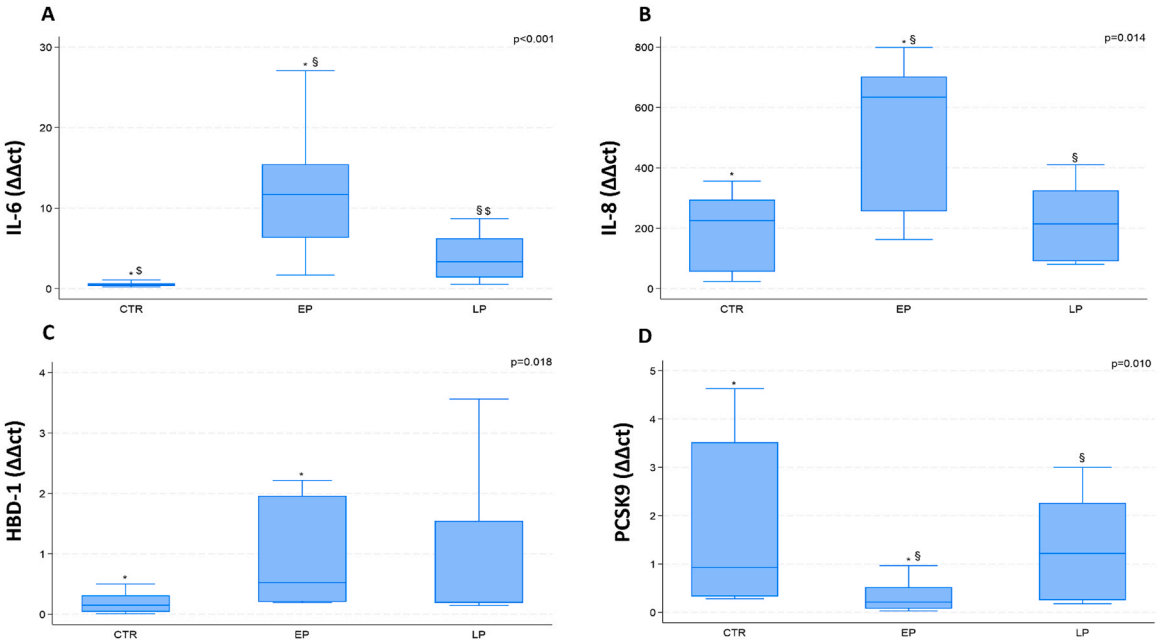

Fig. 1. (A) - Gene expression of IL-6: * (CTR vs EP, $p < 0.001$), § (EP vs LP, $p = 0.005$), $ (CTR vs LP, $p = 0.001$); **(B)** - Gene expression of IL-8: * (CTR vs EP, $p = 0.012$), § (EP vs LP, $p = 0.012$); **(C)**- Gene expression of HBD-1: * (CTR vs EP, $p = 0.010$); **(D)** - Gene expression of PCSK9: * (CTR vs EP, $p = 0.010$), § (EP vs LP, $p = 0.012$).

Table 5
Median and interquartile interval (25th and 75th percentile) values for HNP1, HBD1 and HBD4 in three different group: control group (CTR), early preeclampsia pregnancy group (EP) and late preeclampsia pregnancy group (LP); p-value for Kruskal-Wallis test; * *p-value < α/3 for Bonferroni multiple testing correction.

	Total	CTR	EP	LP	p-value
	N = 30	N = 10	N = 10	N = 10	
HNP1	153.3 (101.2 −191.5)	161.2 (101.2 −170.0)	118.8 (88.1 −148.1)	190.8 (119.0 −244.0)	0.076
HBD1	76.8 (58.4 −138.7)	* *67.8 (58.4 −78.4)vs LP	* *68.6 (49.8 −77.5)vs LP	278.8 (138.7 −336.0)	0.002
HBD4	8.4 (8.0 −9.1)	8.3 (7.9 −8.9)	9.9 (8.4 −12.2)	8.3 (8.0 −8.4)	0.126

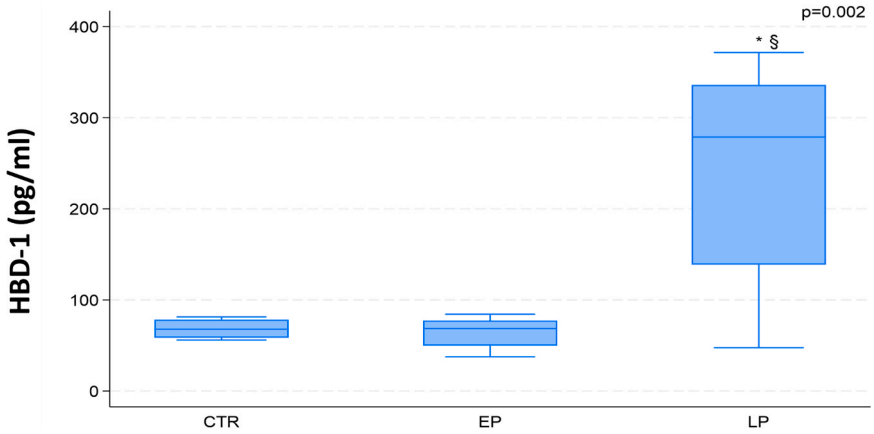

Fig. 2. Serum concentration of HBD-1 in groups: * (LP vs CTR, p = 0.005) § (LP vs EP, p = 0.001).

(p = 0.650); while there are no alterations of HBD-4 in the serum of women affected by PE compared to CTR.

4. Discussion

PE remains an unresolved problem in modern obstetrics. Several biomarkers have been evaluated in both long and short term prediction of the disease; the most used in clinical practice are placental growth factor, soluble Flt-1 and their ratio [33]. Currently, screening of PE in the first trimester is based on a multiparametric screening test, including maternal history, mean arterial pressure, uterine artery velocimetry and placental growth factor. This screening has a detection rate of 89 % for EP, but it fails in detecting LP. Moreover, its implementation has been limited since its cost–effectiveness is still debated [34].

Considering that PE is still a major cause of maternal and neonatal mortality and morbidity in both developed and underdeveloped countries, finding diagnostic, prophylactic and therapeutic tools has been a great obstetric challenge over years [35,36]. We focused to identify the alterations of biochemical parameters and the immune system activity in order to build a panel of markers that can support PE diagnosis through a multidisciplinary approach, based on biochemical methods and molecular biology techniques. Cholesterol is an essential component for foetal and placental development, in fact, in pregnancy, maternal total cholesterol levels increase due to an increased synthesis in the liver [37]. For this reason, we have analyzed the parameters of the lipid profile in women with PE compared to the controls. Serum concentrations of total cholesterol, LDL and HDL are lower in women with PE then controls. Our results are in accordance with Pecks et al., 2012 that found a moderate decrease of LDL in women with PE, assigning this phenomenon as a consequence of hydrolysis and a massive exclusion of LDL-c from the maternal and fetal circulation, but the mechanism is still not clear [38]. Scientific evidence related to Lp(a) levels in women with PE is controversial. Several studies have reported that there is no significant variation in Lp(a) between healthy women and women with PE [39–41], while Bar et al. and Mori et al. reported that Lp(a) increases in PE [42,43]. These variations are probably due to nutrition rather than the presence and/or absence of the disease. Future studies are needed to investigate the role of nutrition and lipid metabolism of PE [44,45]. In our case we found no significative alterations of Lp(a) in populations under study.

In healthy pregnancies PCSK9 levels are higher causing an increase in LDL-R degradation and a subsequent increase in serum LDL-c levels [46]. Our study shows that PCSK9 gene expression is lower in women with PE compared to controls, and in EP compared to LP, underling a greater need for placental salvage of lipids. These results may be associated with states of distress due to PE. Vaught et al. demonstrated that lower maternal PCSK9 levels are involved in LDL-c transport from the placenta to maternal circulation that is insufficient in preeclampsia [47].

Immune system is responsible for protecting against pathogens and, at the same time, induces tolerance to the semi-allogeneic development of the foetus and placenta. An imbalance of pro-inflammatory and anti-inflammatory immune cells and cytokines causes an improper immune response, which leads to the development of PE [48].

In the study population, we highlighted an increase in all pro-inflammatory cytokines (TNF-α, TGF-β, IL-2, IL-6 and IL-8). Among these, IL-6 is an important pro-inflammatory cytokine that regulates acute and chronic inflammatory responses and has distinctive roles in driving inflammatory processes, autoimmunity, and endothelial cell dysfunction. IL-8, instead, is one of the main chemokines for neutrophils and T lymphocytes. From this perspective, the increased IL-8 levels in placenta might play a role in the pathogenesis of PE through enhanced recruitment of neutrophils, which, in turn, increases local inflammation [49]. In this scenario, IL-6 and IL-8 have showed a significant increase in EP compared to controls and LP, proving that early condition is more severe than late preeclampsia. For this reason, IL-6 and IL-8 could also be considered biomarkers of the early development of the disease [50].

On the other hand, although gene expression of HBD1 increased in both EP and LP compared to controls, HBD1 serum levels increase in LP compared to EP and controls, showing a protective role in inflammation and/or infection [51,52]. As a consequence, women with higher HBD1 levels are protected from the onset of severe forms of PE and the development of the pathology is slightly delayed.

Following these results, it is certainly possible to state that during PE there is a hyper-activation of the immune system caused by a violent

cytokine cascade; but on the other hand, it may happen that the same immune system on alert, can trigger a series of protective actions to prevent adverse outcome.

PE is characterized by activation of both pro-inflammatory and anti-inflammatory mechanisms and the last ones could be a protective response of the immune system to reduce the risk of adverse outcomes. Moreover, in PE there is a reduction in LDL-c levels and gene expression levels of PCSK9 causing a lower supply of lipid precursors necessary for the synthesis of placental hormones and foetal fatty acids. PCSK9 could be considered a biomarker associated with state of distress and indicate a greater need for placental and foetal salvage of lipids in PE.

It would be of research interest, to understand if the differences we highlighted among CRT, EP, and LP could have been found already in the first trimester, in order to understand if the reported molecules, or some of them, can be used as possible early markers of PE. Indeed, it is well known that, nowadays, we can only offer a prophylactic treatment with Aspirin to prevent PE and that this treatment should be started early in pregnancy, in general before 16 weeks of gestation [33]. Therefore, it is important to find early biomarkers that could help identifying "at risk" pregnant women. Further studies assessing a possible role of these factors in the first trimester are, therefore, strongly encouraged.

Moreover, further studies with a larger number of patients are needed to better clarify the role of immune system in this pathology.

In conclusion our results can help understanding pathophysiological mechanisms underlying the disease and they reveal a new scenario from a diagnostic and therapeutic point of view. In fact, the use of personalized medicine and the use of specific drugs could be an avant-garde therapy in the case of PE in pregnancy.

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Declaration of Competing Interest

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Author contributions

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