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Systemic and placental α -klotho: Effects of preeclampsia in the last trimester of gestation

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Abstract

Introduction— α -klotho is an anti-aging protein, potentially important in preeclampsia (PE). Produced by kidney, brain and placenta, and by mRNA splicing is both a full-length membrane-bound and a truncated soluble protein in the circulation. The membrane-bound protein is an obligate co-receptor for fibroblast growth factor 23 (FGF23) and its action on receptor (FGFR), but ADAM proteinases also cause its shedding. The aims of this study were to investigate levels of maternal plasma, placental, and fetal membrane α -Klotho and their association with placental accelerated villous maturation (AVM) in PE. In addition, placental and membrane levels of ADAM17 and FGFR were measured in the same patients.

Methods—Maternal blood, placenta and fetal membranes from 61 women (31 with PE and 30 controls) between 32 and 40 weeks gestation were collected. Plasma α -klotho was measured by ELISA, and quantitative immunohistochemistry used for α -klotho, ADAM17 and FGFR1 in tissues. Placental AVM was histologically assessed.

Results—Maternal plasma levels of α -Klotho were higher in PE compared to controls ($p = 0.01$) and patients with the highest levels had significantly less AVM ($p = 0.03$). α -Klotho, ADAM17, and FGFR were all present in syncytiotrophoblast and cytotrophoblast of membranes. Between 32 and 40 weeks gestation, all placental levels decreased in controls respectively ($p = 0.04$, $p = 0.004$, $p = 0.05$), but not in PE. Fetal membrane levels were unchanged.

Discussion—Maternal plasma α -Klotho was increased in PE and its levels associated with reduced placental AVM. Changes in placental α -Klotho, ADAM17, and FGFR suggest their involvement in the pathophysiology of PE.

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Conflict of interest

All the authors have no conflict of interest.

Keywords

Klotho; Preeclampsia; Pregnancy; Hypertension; Accelerated villous maturation

1. Introduction

Hypertensive disorders of pregnancy are still a significant cause of maternal and perinatal morbidity and mortality. Preeclampsia (PE) complicates 10% of all pregnancies and is responsible for 20% of maternal deaths in the United States [1]. Several mechanisms, such as abnormal placentation [2,3], oxidative stress [4,5], placental ischemia [6] and endothelial dysfunction [7,8] are all pathophysiologic aspects of PE. The placenta has a central role, producing bioactive factors, which act both locally and in the maternal compartment, with the potential for involvement in the genesis of hypertension [9]. An inadequacy in the placenta is the most likely reason for the increased placental oxidative stress, which in turn generates greater cellular stress, causing inflammatory effects within and beyond the placenta [10]. Prolonged oxidative damage in the placenta gives rise to characteristic placental histology [11,12], defined as accelerated villous maturation (AVM) correlated with PE [11,12] and preterm birth [13]. However, there are few studies to date which attempt to link placental histological profiles with upstream molecular events.

α -Klotho is an anti-aging protein, which when disrupted in mouse models gives rise to a syndrome closely resembling human aging. Transgenic knockout mice show skin atrophy, atherosclerosis, osteopenia, cognitive impairment and motor neuron degeneration at 3–4 weeks and die prematurely at two months [14]. At the cellular level, α -klotho regulates senescence by repressing the p53/p21 pathway [15]. In humans, a functional variant of the *Klotho* gene has been shown to be associated with human survival [16]. In cross-sectional and prospective studies in human populations, *Klotho* has been implicated in the etiology of cardiovascular disease and mortality [17]. α -klotho is produced in the kidney and brain and to a lesser degree the placental syncytiotrophoblast [18,19]. The klotho gene generates both a full-length membrane-bound protein and a truncated soluble secreted (systemic) form by alternative mRNA splicing [20]; the major human gene product is the secreted protein [21]. However, the trans-membrane protein is also shed into blood, urine and cerebrospinal fluid [21]. This is accomplished by cleavage and release by the disintegrin and metalloproteinases; ADAM10 and 17 [22]. ADAM17 is a major sheddase for placental TNF α , and increases in preeclampsia [23]. The receptor for soluble α -klotho is unknown, whereas the trans-membrane form interacts with three fibroblast growth factor receptor (FGFR) isoforms (FGFR 1c, 3c and 4). It serves as an obligate co-receptor for bone derived fibroblast growth factor 23 (FGF23), which is secreted from bone into the circulation [24,25], but is not produced by the placenta [26]. Thus, α -klotho significantly increases the affinity of specific FGFRs for FGF23 [27].

α -Klotho functions in several biological processes involved in the pathophysiology of PE including endothelial nitric oxide production, angiogenesis, antioxidant enzyme production and protection against endothelial dysfunction [28–30]. Maternal soluble α -klotho is higher in pregnant compared to non-pregnant women. However, PE together with a small-for-

gestational age infant was associated with lower than normal levels [31]. There have been no studies to date, which concomitantly measured maternal α -klotho and placental α -klotho, ADAM17 and FGFR1, in placenta and fetal membranes of a single set of patients. In this study, we therefore aimed to gain new insights into the α -Klotho system in the maternal circulation and in the placenta in PE.

2. Materials and methods

2.1. Study participants and sample collection

PE was defined according to current guidelines from the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy [32]. All patients were preterm (≥ 36 weeks 6 days) or term (≥ 37 weeks 0 days). Controls had no medical comorbidities, no illicit drug or tobacco use. Preterm controls had spontaneous preterm labor or preterm premature rupture of membranes (PPROM) and were delivered during the same admission. These cases accounted for the majority of preterm controls (12/15 = 80%). In cases of preterm PPRM ≥ 34 weeks, labor was augmented with Pitocin in accordance with ACOG recommendations. The other three preterm controls were admitted for scheduled cesarean delivery due to placenta previa (3/15 = 20%). Preterm neonates were classified as small-for-gestational-age (SGA) using gender specific Fenton growth curves [33]. There are currently no validated ethnic-based fetal/neonatal growth curves and ten percent of our controls were SGA, likely due to our high prevalence of Asian patients, specifically Japanese and Filipino [34].

After approval from Hawaii Pacific Health and Western Institutional Review Board, pregnant women admitted to Kapi'olani Medical Center for Women and Children (Honolulu, HI) meeting study criteria were recruited with informed consent in 2013–2014. Exclusion criteria included maternal connective tissue/autoimmune disease, renal disease, pre-gestational or gestational diabetes mellitus, active infection, chronic corticosteroid use, illicit drug or tobacco use, anemia (hemoglobin < 10 mg/dl), obstructive sleep apnea, multiple gestation, uterine malformations, fetal chromosomal/congenital anomalies, or histologic evidence of placental infection. Prior to delivery, maternal blood samples were collected and plasma stored at -80°C . Immediately after delivery, one full-thickness sample of placenta, excluding basal plate, was collected near the cord insertion site, fixed in neutral-buffered formaldehyde for 72 h and embedded in paraffin.

2.2. Maternal plasma analyses

Maternal plasma sFlt-1 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). Inter-assay and intra-assay coefficients of variation were 7.4% and 3.2% respectively with sensitivity of 3.5 pg/mL. Significantly higher levels in PE compared to controls were found ($15,599.0 \pm 11,949.7$ pg/mL vs. 3423.2 ± 2417.3 pg/mL; $p < 0.0001$) consistent with other studies. α -Klotho was measured by ELISA (Immuno-Biological Laboratories Inc., Minneapolis, MN) with inter-assay and intra-assay coefficients of variation of 4.7% and 2.9% and sensitivity of 6.16 pg/mL. All samples were assayed in duplicate, with personnel blinded to clinical information and outcomes.

2.3. Placental histopathology, immunohistochemistry and quantitation

Histologic evaluation was performed by a placental pathologist (KT) blinded to gestational age and clinical outcomes. H&E stained placental sections were evaluated for AVM (narrow terminal villi and/or syncytial knotting out of proportion for gestational age) and scored positive if the finding was present in multiple 10× objective fields [11,12,35].

Immunohistochemistry was performed for quantitative assessment [36], using serial sections (5 µm) heated in sodium citrate buffer (10 mM, pH 6.0) for 20 min for antigen retrieval. The Vectastain Elite kit (Vector Labs, Burlingame, CA) was used according to the manufacturer's protocol. Non-specific binding was blocked with 2.5% normal serum for 20 min before incubation for 60 min with antibodies: polyclonal goat IgG to α -klotho (Santa Cruz Biotechnology, Santa Cruz, CA, sc-22218, 0.75 µg/mL), polyclonal rabbit IgG to FGFR1 (Santa Cruz Biotechnology, sc-121, 0.25 µg/mL), or monoclonal mouse IgG2b to ADAM17 (ABCAM, Burlingame, CA, ab 57484, 1.25 µg/mL). Negative controls were species-specific non-immune IgG at equivalent concentrations with 3,3-diaminobenzidine (DAB) substrate solution used for visualization. Slides were rinsed, counterstained with Gill's hematoxylin, cleared and mounted.

A multispectral imaging system with an Olympus BX51 microscope (Olympus America Inc, Mellville, NY) and a CRI Nuance spectral analyzer (Caliper Life Sciences, Hopkinton, MA) was used to obtain brightfield image cubes between 420 and 700 nm wavelength at 20 nm intervals. The Inform Tissue Finder software (version 2.0.2; PerkinElmer, Waltham, MA) segmented tissues into cell types, unmixed the spectral components, and quantified staining levels [37]. Average signal intensity per pixel, was obtained from five different fields from each patient, as mean optical density units (ODU). Signal intensities for α -klotho, FGFR1, and ADAM17 were quantified at 400× magnification. Laboratory personnel were blinded to clinical information.

2.4. Statistical analysis

Demographic and clinical information were summarized (means $\pm$ SD) for continuous variables, frequencies and percentages for categorical variables. Groups were analyzed by Fisher's exact test and the Kruskal-Wallis test for continuous variables. For α -klotho, comparisons were made by one-way analysis of variance (ANOVA) with Tukey's post-hoc analysis. Associations of α -klotho, ADAM17, and FGFR1 with gestational age were measured by linear regression models with slopes compared between controls and PE. Data analysis used SAS statistical software version 9.3 (SAS Institute Inc., Cary, NC). Two sided p-values ≤ 0.05 were considered statistically significant. All significant findings remained significant after multivariable adjustments.

3. Results

3.1. Patient characteristics

PE is a multi-system disorder with marked heterogeneity in its clinical manifestation, possibly due to different molecular pathogenesis. This study used fresh samples, deemed important for analysis of a fragile protein about which, little is known during human gestation. In order to do this, we had to include a less than ideal heterogeneity in the patient

demographics. However, for each set of results obtained, we separately analyzed the following groups: preterm (early onset) and term (late onset) PE, PE patients with and without HELLP syndrome, and labor/non-labor. There were no significant differences found in any of the proteins measured in these sub-groups in either the plasma, placenta, or fetal membranes. Therefore, we used the data in two groups, those with PE and those without (controls).

Patient demographics are shown in Table 1. For patients with PE, 9 (29%) had HELLP, 18 (58.1%) delivered preterm and 13 (41.9%) term. For controls, 15 (50%) delivered preterm and 15 (50%) term. There were no significant differences in maternal or gestational ages or chronic hypertension in women with PE and controls. As expected, women with PE had significantly fewer pregnancies, fewer prior deliveries, earlier gestational age, lower birth weight, higher blood pressure and rates of labor, and higher rates of small for gestational age neonates compared to controls.

3.2. α -Klotho in placenta, fetal membranes and maternal plasma of controls

Little is known about α -klotho in the placenta, fetal membranes and maternal plasma in gestation. Therefore, this was addressed in control samples between 32 and 40 weeks. In placenta, α -klotho was immunolocalized predominantly to the syncytiotrophoblast brush border and, to a much lesser degree in the cytotrophoblast, shown in examples at preterm (Fig. 1A) and term (Fig. 1B), the negative control had no staining (Fig. 1C). Only a few cytotrophoblast cells were evident in our samples at this period of gestation. In the fetal membranes, immunostaining was predominantly in the chorionic cytotrophoblast, and lighter in decidual cells, shown at preterm (Fig. 1D) and term (Fig. 1E). In the latter, there was variation in staining intensity of the individual chorionic cytotrophoblasts, not evident at preterm. The negative control had no staining (Fig. 1F). In maternal plasma, α -klotho increased slightly but not significantly, whereas placental α -klotho significantly decreased ($p = 0.04$) (Fig. 1G). In fetal membranes, there was significantly more α -klotho in the cytotrophoblast compared to decidua ($p < 0.0001$) (Fig. 1H). However, quantitation in the decidua may have been over-estimated since a few extravillous trophoblast cells would have been included in the decidual layer.

3.3. α -Klotho in maternal plasma, placenta and fetal membranes in preeclampsia

Maternal plasma α -klotho levels were significantly higher ($p = 0.01$) in patients with PE compared to controls (2019 ± 1320 pg/mL vs. 1277 ± 909 pg/mL respectively, Fig. 2A). Levels increased slightly in controls and decreased slightly in PE between 32 and 40 weeks, neither were significant (Fig. 2B). Placental α -Klotho had the same distribution of immunolocalization in PE as controls and syncytiotrophoblast quantitation showed no significant difference (Fig. 2C). However, as a function of gestational age, levels in PE failed to decrease as they did in controls (Fig. 2D). There were no significant differences in the chorionic cytotrophoblast or decidua in PE compared to controls (data not shown).

3.4. Placental and fetal membrane ADAM17

Immunolocalization of ADAM17 in placentas showed prominent staining in syncytiotrophoblast, agreeing with Ma et al. [23]. This was darker at preterm (Fig. 3A)

compared to term (Fig. 3B), with no staining in the negative control (Fig. 3C). In fetal membranes, ADAM17, was predominantly localized to chorionic cytotrophoblast and decidua. Examples of preterm (Fig. 3D) and term membranes (Fig. 3E) and negative control are shown (Fig. 3F). Quantitation in placenta showed significantly more in PE compared to controls ($p = 0.008$, Fig. 3G). Analysis by gestational age (Fig. 3H), showed control levels significantly declined ($p = 0.004$), whereas in PE they remained elevated, similar to placental levels of α -klotho (Fig. 2D).

3.5. Placental and fetal membrane FGFR1

Immunolocalization of FGFR1 showed prominent staining in placental syncytiotrophoblast, agreeing with Ohata et al. [26] and was also darker at preterm than term, examples shown (Fig. 4A and B) with a negative control (Fig. 4C). In fetal membranes, the chorionic cytotrophoblast stained predominantly and was lighter in decidua. Examples of preterm (Fig. 4D) and term membrane (Fig. 4E) with a negative control (Fig. 4F). Quantitation in syncytiotrophoblast showed no significant difference in PE and controls (Fig. 4G). However, by gestational age, levels significantly decreased in controls ($p = 0.05$) but not in PE ($p = 0.5$) (Fig. 4H), similar to both placental α -klotho (Fig. 2D) and ADAM17 (Fig. 3H).

3.6. α -Klotho and placental accelerated villous maturation

Examples of H&E stained placenta with features of AVM are shown in Fig. 5A–D: small terminal villi with decreased amounts of stroma and longitudinal forms with reduced branching in a preterm PE (Fig. 5A, B), and accentuated syncytial knotting was evident at term PE (Fig. 5C, D). A term control lacked any of these features (Fig. 5E, F). The frequency of AVM in PE was significantly higher than controls ($22/31 = 71\%$ vs. $12/30 = 40\%$, $p = 0.02$). In PE, maternal α -klotho was significantly higher ($p = 0.03$) when AVM was absent (Fig. 5G). However, there was no significant difference in maternal α -klotho with/without AVM in controls (Fig. 5G). On the other hand, placental α -klotho levels were not significantly different in controls or PE with/without AVM (Fig. 5H).

4. Discussion

Maternal plasma sFlt-1, a marker of PE, was significantly elevated in our samples showing them to be consistent with other studies [38]. We confirmed expression of α -klotho, ADAM17 and FGFR1 in placenta, extending this to chorionic cytotrophoblast and decidua of fetal membranes. This preliminary study shows that there were increased levels of maternal plasma α -Klotho in PE, which were associated with reduced placental AVM. At the same time, placental FGFR1 failed to decline over the last trimester of gestation in PE as it did in controls. A limitation of this study was the heterogeneity of our sample set. However, we separately analyzed all of our data for a number of important variables (see Results). We found no significant effects of these on the proteins measured, but the resulting small numbers point to the need for future studies with larger sample size to take those factors into account.

There is a paucity of information on α -klotho in the maternal circulation or placenta during normal human gestation. Our controls, between 32 and 40 weeks showed a slight increase in

soluble α -klotho, agreeing with a recent study [31]. However, we show a significant decline in placental α -klotho, suggesting either less production as gestation advanced or more rapid shedding into maternal and/or cord blood. Concomitantly, there were significant declines in both ADAM17 and FGFR1. Thus, in controls over this period, there appeared to be a coordinated lowering of the placental α -klotho system, suggesting both less shedding of α -klotho and a parallel reduction as a co-receptor with FGF23 to activate FGFR1 in syncytiotrophoblast [27]. However, due to α -klotho dilution in the maternal circulation and other major tissue sources [18] more work is needed to confirm this.

Antibodies previously evaluated by others for immunolocalization in the placenta, confirmed predominant syncytiotrophoblast localization of α -klotho [19] ADAM17 [23] and FGFR1 [26]. The lack of placental production of *fgf23*FGF23 suggested its presence in maternal blood would suffice for placental α -klotho to act as a co-receptor for FGFR1 in syncytiotrophoblast [26]. In both mouse and human, FGF23 functions as an endocrine hormone, the resulting complex of FGF23, α -klotho and FGFR1 are involved in vitamin D, phosphate and calcium metabolism in the syncytiotrophoblast [26]. We show here that the chorionic cytotrophoblast of the fetal membranes, express the components of this system. However, lack of changes in controls or in PE, does not preclude its potential importance in other pregnancy complications.

The pathophysiology of PE altered placental levels of α -klotho, ADAM17 and FGFR1, and significantly increased maternal α -klotho. A previous study showed no change in its maternal levels with PE or PE and SGA [31]. However, this study showed that α -Klotho levels in the maternal circulation patients with SGA and no PE were significantly lower than their controls. Our study focused on PE alone; the small number of patients with SGA neonates in our cohort precluded a separate analysis of SGA.

A recent study has shown that the levels of soluble α -klotho are markedly elevated in human umbilical cord blood compared to its levels in the maternal circulation or in neonates at day 4 [19]. These authors suggested that the syncytiotrophoblasts in the placenta were likely to be one of the major sources. In our study, in PE we have found increased soluble α -klotho in the maternal circulation, without any increase in the placental syncytiotrophoblast. The α -klotho protein was located predominantly on the syncytiotrophoblast microvillous membrane, well positioned for shedding. We also found that the syncytiotrophoblast ADAM17 levels were increased, confirming a previous study [23], which would increase shedding. From the work of Ohata and colleagues on cord blood [19] and our observation here in PE, the placenta may be one of the major sources of soluble α -klotho. However, further work is needed to show this. Since soluble α -klotho in the human is its major protein form [20], it would be important to show that in pregnancy, some or most of this indeed originates from the syncytiotrophoblast. In addition, in order to more fully understand its role(s) in the mother, placenta and fetus, its actions, in both normal and pathological pregnancies require further study.

During normal gestation, increased oxidative stress by late gestation manifests as histologically increased frequency of true syncytial knots or Tenney-Parker change [39], with nuclei in these knots showing signs of oxidative damage, considered to be related to

normal placental aging [40]. However, the formation of syncytial knots is increased in PE and is attributed to premature aging of the placenta in this pathology [11,12,40]. α -klotho is an anti-aging protein, therefore we sought an association with AVM. It was surprising that placental α -klotho levels were unrelated to AVM. However, maternal plasma levels of α -klotho were higher in the PE patients without AVM. Thus, lower maternal α -klotho levels, shown to cause increased p53/p21, might mediate increased cellular senescence [15] manifesting as placental AVM. Indeed, most nuclei in true syncytial knots are transcriptionally inactive, but further work is required to show that soluble α -klotho is indeed involved in these histological changes. In addition, the ontogeny of α -klotho in the placental tissues from early first trimester to term and its relationship with the development of AVM requires further study.

In conclusion, soluble α -klotho levels were increased in maternal plasma in PE, with the highest levels associated with reduced placental AVM. Changes in placental α -klotho, ADAM17 and FGFR, suggest their potential involvement in the pathophysiology of PE.

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Abbreviations

PE	preeclampsia
HELLP	hemolysis elevated liver enzymes, and low platelets
sFlt-1	soluble vascular endothelial growth factor receptor-1
SGA	small for gestational age
ELISA	Enzyme-linked immunosorbent assay
FGFR	fibroblast growth factor receptor
FGF23	bone derived fibroblast growth factor 23
AVM	accelerated villous maturation
ADAM	a disintegrin and metalloproteinase

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Fig. 1.

α -klotho in plasma, placenta, and fetal membranes in control patients (32–40 weeks). A–C. Examples of placentas immunostained for α -klotho (A) preterm (B) term (C) negative control. α -klotho localized to the syncytiotrophoblast (ST) and cytotrophoblast (CY). D–F. Examples of fetal membranes: (D) preterm (E) term (F) negative control. α -klotho localized to the chorionic cytotrophoblast (CC) and decidua (dd) with darker staining in the chorion. G. maternal plasma α -klotho (dotted line, open circles) slightly increased with gestational age, placental α -klotho (solid line, closed triangles) significantly decreased ($p = 0.04$). H. Chorionic cytotrophoblast (dotted line, open circles) and decidua (solid line, closed triangles), significantly more in the chorion compared to decidua ($p = 0.0001$). Original magnification 400 $\times$.

Fig. 2.

Maternal and placental α -klotho in PE and controls (32–40 weeks). A. maternal plasma α -klotho was significantly higher in patients with PE ($p = 0.01$). B. as a function of gestational age (solid line, closed triangles) showed no significant change. Controls (dotted line from Fig. 1G for comparison). C. Placental α -klotho was not significantly different in PE. D. as a function of gestational age, α -klotho failed to decline in PE (solid line, closed triangles) compared to controls (dotted line from Fig. 1G for comparison).

Fig. 3. ADAM17 in placenta and fetal membranes in PE and controls (32–40 weeks). A–C. Examples of placentas immunostained for ADAM17 from (A) preterm (B) term (C) negative control. ADAM17 localized to the syncytiotrophoblast (ST). D–F. Examples of fetal membranes from controls (D) preterm (E) term (F) negative control. ADAM17 localized predominantly to the chorionic cytotrophoblast (CC) and in some cells of decidua (dd). G. Placental ADAM17 was significantly higher in PE ($p = 0.008$). H. as a function of gestational age, showed a significant decrease in controls ($p = 0.004$; dotted line, open circles) and no decline in PE (solid line, closed triangles). Original magnification 400 $\times$.

Fig. 4. FGFR1 in placenta and fetal membranes in PE and controls (32–40 weeks). A–C. Examples of placentas immunostained for FGFR1 from controls (A) preterm (B) term (C) negative control. FGFR1 localized to the syncytiotrophoblast (ST). D–F. Examples of fetal membranes from controls (D) preterm (E) term (F) negative control. FGFR1 localized to the chorionic cytotrophoblast (CC) with light staining in decidua (dd). G. Placental FGFR1 was not significantly different from controls. H. as a function of gestational age it significantly decreased in controls ($p = 0.05$; dotted line, open circles) but failed to decline in PE (solid line, closed triangles). Original magnification 400 $\times$.

Fig. 5. α -Klotho and placental accelerated villous maturation (AVM). A–D show H&E stained placentas from PE consistent with AVM. A. Preterm: Low power view reveals small terminal villi (TV, green arrows) with decreased amounts of stroma, longitudinal forms (LF, black arrows), and reduced branching (100 $\times$). B. High power section highlights small tertiary villi with a single capillary or absent capillaries (arrows, 200 $\times$). C. Term: Villi are not as small or thin as in the preterm placenta, and accentuated Tenney-Parker change (TP, black arrows) can be appreciated on low power (100 $\times$). D. High power magnification highlights the accentuated syncytial knots with villous adhesion, characteristic of TP (arrows, 200 $\times$). E. Normal term control showing wider villi with normal branching (100 $\times$). F. Syncytial knotting is not accentuated (200 $\times$). G. Maternal α -klotho was significantly higher in PE when there was no AVM ($p = 0.03$), controls showed no significant difference. H. Placental α -klotho was not different in patients with PE or controls with or without AVM.

Table 1

Patient demographics.

Variables	Controls n = 30	PE n = 31	<i>p</i> -value
Mean age, years (mean $\pm$ SD)	29.6 $\pm$ 6.1	28.0 $\pm$ 6.9	0.51
Gravidity (mean $\pm$ SD)	3.5 $\pm$ 2.3	2.1 $\pm$ 1.4	0.003
Parity (mean $\pm$ SD)	1.6 $\pm$ 1.3	0.7 $\pm$ 1.1	0.002
Mean systolic BP, mmHg (mean $\pm$ SD)	116.0 $\pm$ 9.2	163.7 $\pm$ 17.6	<0.0001
Mean diastolic BP, mmHg (mean $\pm$ SD)	68.9 $\pm$ 9.0	96.8 $\pm$ 9.8	<0.0001
Labor, n (%)	12 (40.0)	26 (83.9)	0.0004
Vaginal delivery, n (%)	12 (40)	20 (64.5)	0.07
Cesarean delivery, n (%)	18 (60)	11 (35.5)	0.07
Chronic hypertension, n (%)	0	3 (9.7%)	0.10
Mean gestational age at delivery, weeks (mean $\pm$ SD)	37.1 $\pm$ 2.3	36.2 $\pm$ 2.0	0.07
Small for gestational age neonate, n (%)	3 (10.0)	9 (29.0)	0.06
Mean neonatal weight, g (mean $\pm$ SD)	2868.4 $\pm$ 649.3	2449.0 $\pm$ 574.0	0.002