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Original Article

Clinic significance of markedly decreased α-klothoin women with preeclampsia

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Abstract: Preeclampsia (PE) is a leading cause of maternal and perinatal morbidity and mortality. Klotho is a novel gene and the secret form, α-klotho (α-KL), is related to preeclampsia. We conducted this cross-sectional study in Wuhan, China. We used immunohistochemistry, real-time PCR, western blot, ELISA to measure α-KL expression in placenta and its secretion in maternal and umbilical cord serum, and analyzed correlations between α-KL level and other parameters in normal and preeclampsia pregnancy. We found that both mRNA and protein expression of placental α-KL in women with PE was significantly lower than that in normal pregnancy. Also, expression level of α-KL in both maternal and umbilical cord was markedly decreased in PE patients. Further analyses showed that serum α-KL exhibited positive association with fetal birth weight, and reverse association with oxidative stress and renal function markers. Receiver operating characteristic analysis suggested α-KL might be a potential predictor for preeclampsia.

Keywords: Preeclampsia, α-klotho, fetal birth weight, oxidative stress, renal function, receiver operating characteristic

Introduction

Preeclampsia (PE), mainly occurs in women with first or multiple pregnancies, is a leading cause of maternal and perinatal morbidity and mortality. There are many risk factors for PE such as hypertension, diabetes mellitus, obesity, family history of PE, null parity, multiple pregnancies and thrombotic vascular disease [1]. If PE remains untreated, it can develop towards eclampsia, producing maternal death. Additionally, PE predisposes fetus to future cardiovascular disease and other disorders [2].

Klotho (KL) was initially identified as a novel anti-aging gene in mice. It encodes a type I single transmembrane protein that shares 86% of amino acid sequence with the human KL protein [3]. Experiments on mice demonstrated that over-expression of KL extended life span, increased resistance to insulin and oxidative stress *in vivo* and *in vitro* [4].

In humans, KL is predominantly expressed in kidneys, parathyroid glands, adipose tissue and choroid plexus, but it is also distributed in other tissues such asprostate, small intestines, placenta and umbilical cord blood [5]. KL has two transcripts, trans-membrane and secreted form. The secreted form of KL (α-KL), which is found in blood, urine, and cerebrospinal fluid, expresses predominately over the trans-membrane form [6]. α-KL plays a vital role in the development of age-related diseases in mammals such as acute kidney injury (AKI), chronic kidney disease (CKD), cardiovascular disease (CVD) and cancer [7]. Previousstudies revealed several plausible explanations: (1) α-KL acts as a circulating hormone in the circulation system and modulating the response of inflammation; (2) α-KL regulates fibroblast growth factor (FGF) 23 which functions in the kidney; (3) α-KL protects against endothelial dysfunction and regulates the production of nitric oxide [6]. There were also studies that linked α-KL with PE. Giannubilo *et al.* found that α-KL mRNA expression in the placenta was decreased in PE, and α-KL may link to long term outcomes in PE mothers and their off spring [8], suggesting that

further research on α-KL and PE was meaningful. Recently, Miranda *et al.* demonstrated that the median maternal plasma concentration of α-KL was lower in mothers who delivered a small-for-gestational age (SGA) infant than in those with an uncomplicated pregnancy no matter whether mothers had PE or not [9]. In their study, maternal pre-pregnancy body mass index (BMI) was not considered as a controlled variable. Previous studies suggested that obese women were more likely to deliver at earlier gestational ages and pre-pregnancy BMI is highly correlated with fetal birth weight [10]. Therefore, we controlled maternal pre-pregnancy BMI in the present study and measured α-KL expression in placenta, maternal and umbilical cord serum, and explored the associations between α-KL and fetal birth weight as well as maternal renal function, and determined whether α-KL level was correlated with oxidative stress in PE.

Methods

Study design

We conducted the cross-sectional study in School of Public Health, Wuhan University and Renmin Hospital, Wuhan University. Two groups of women were recruited: (1) women without an uncomplicated pregnancy (NC); (2) women with PE (PE). Women with overweight/obesity, multiple gestations, chronic hypertension, diabetes, renal diseases, and fetuses affected with chromosomal and/or congenital anomalies were excluded. Finally, 42 pregnant women with 23 normal pregnancy and 19 severe PE were enrolled consecutively in Renmin Hospital, Wuhan University from July to December, 2012. After being informed of the purpose and procedures of the study, all participants signed an informed consent form. The study protocol was approved by the Ethics Committee of Wuhan University.

Clinical definitions

Patients were diagnosed to have PE if they met the following criteria: hypertension (systolic blood pressure higher than 140 mmHg or diastolic blood pressure higher than 90 mmHg on at least two occasions, 4 to 6 hours apart) presents after 20 weeks of gestation combined with proteinuria (> 300 mg/24 h) or random proteinuria ≥ (+), other maternal organ dysfunction such as liver involvement, neurological or hematological complications, renal insufficiency [2]. Severe PE was defined as: (1) systolic blood pressure of at least 160 mmHg and/or diastolic blood pressure of at least 110 mmHg on two occasions; (2) proteinuria ≥ 5.0 g/24 h or random proteinuria ≥ (+++); (3) thrombocytopenia (two platelet counts < 100,000/mm^3) [11].

Baseline data and sample collection

The basic information of all participants was collected by questionnaire, including age, weight, height, gestational age, gestational weight gain, fetal birth weight and Apgar score. The primary biochemical parameters such as total protein (TP), albumin (ALB), serum creatinine (SCr), uric acid (UA) were collected from clinical examination record. 3 ml of maternal peripheralblood samples were collected and 4 ml of umbilical cord blood were obtained from the umbilical cord immediately after delivery. Serum was obtained by centrifugation at 2500 rpm/min for 5 minutes and stored at -80°C.

Immunohistochemistry (IHC)

Placenta tissue samples were fixed with 4% PFA (Paraformaldehyde), and embedded in paraffin, sectioned and stained with klotho antibody (Abcam, 1:200), and counterstained with hematoxylin. Stained image was captured from three slides of each from ten patients per group and the relative density was determined using NIH Image J software.

Real-time PCR

Total RNA was isolated from placenta lysate by Trizol (Invitrogen). RT reactions were performed using standard method (5-min reverse transcriptase inactivation at 95°C, and 40 cycles at 95°C for 15 s, 56°C for 15 s and 72°C for 20 s). cDNA were analyzed by real-time PCR using SYBR Green (ABI) normalized to reference gene b-actin on an Applied Biosystems Stepone Plus system. Gene expression was calculated as 2-ΔΔCt. Primer sequences are: Klotho Forward: 5'-ACT CCC CCA GTC AGG TGG CGG TA-3'; Reverse: 5'-TGG GCC CGG GAA ACC ATT GCT GTC-3'; β-actin Forward: 5'-GGA CTT CGA GCA AGA GAT G-3'; Reverse: 5'-AGC ACT GTG TTG GCG TAC G-3'.

Table 1. Demographic and clinical characteristics of the subjects

Parameters	NC (n=19)	PE (n=23)	*P* value
Age (y)	28.3±3.0	28.6±5.6	0.66
Parity	1.2±0.56	1.2±0.60	0.86
Pre-pregnancy BMI (kg/m^2)	21.3±3.0	21.7±4.0	0.76
Gestational weight gain (kg)	16.97±7.33	16.65±5.58	0.19
Gestational age (week)	39.1±1.6	36.6±2.8	0.008
Fetus birth weight (g)	3439±505	2561±705	0.0002
Apgar score	8.95±0.23	8.09±1.12	0.0000

NC: Normal group; PE: Preeclampsia group.

Table 2. The primary biochemical parameters

Sample type	Parameters	NC (n=23)	PE (n=19)	*P* value
Maternal serum	α-KL (pg/ml)	951.2±323.9	579.0±228.4	0.0000
	TP	63.3±4.4	59.9±7.6	0.126
	ALB	33.5±3.2	28.9±5.3	0.011
	Urea	3.1±0.6	3.8±1.5	0.038
	SCr	46.7±9.1	55.4±11.2	0.016
	UA	320.6±68.6	368.5±104.9	0.1234
	Ca	2.6±0.1	2.1±0.1	0.049
	Corrected Ca	3.0±0.4	2.8±0.2	0.071
Umbilical cord serum	α-KL (pg/ml)	1949±328.3	1617±428.7	0.0000

NC: Normal group; PE: Preeclampsia group; α-KL: α-klotho; TP: Total protein; ALB: Albumin; SCr: Serum creatinine; UA: Uric acid. *: P < 0.05. ALB: Albumin; UA: Uuric acid; SCr: Serum creatinine; Ca: Calcium; TP: Total phosphorus.

Western blot (WB)

Standard immunoblotting procedure was carried out with 100 mg protein per sample from tissues. Briefly, proteins were submitted to SDS-PAGE, transferred to nitrocellulose blotting membranes, and blocked in 5% fat-free milk, and probed with klotho (Abcam, 1:200) and b-actin (Sigma, 1:2500) antibodies. The membranes were visualized by chemiluminescence reagents (SuperSignal West Pico, Pierce) and quantitated with Image J software.

ELISA

We used α-KL ELISA kit (Cusabio, Wuhan, China) to measure α-KL levels in maternal and umbilical cord serum [12]. All samples was detected in duplication.

Malondialdehyde (MDA) activity detection

A Malondialdehyde (MDA) Detection Kit (A003, Nanjing Jiancheng Bio engineering Institute, Nanjing, China) was selected to determine the MDA level. Assay was conducted according to the manufacturer's instructions [13].

Superoxide dismutase activity measurement

The Superoxide Dismutase Detection Kit (S0101, Beyotime Institute of Biotechnology, Shanghai, China) was selected for SOD measurement. The assay was conducted according to the manufacturer's instruction.

Glutathione peroxidase (GPx) activity detection

To determine the activity of GPx from each treatment group, the Glutathione Peroxidase Detection Kit (S0052, Beyotime Institute of Biotechnology, Shanghai, China) was selected. The determination of GPx is based on the manufacturer's instructions [14].

Statistical analyses

Continuous variable are expressed as mean and standard deviation, to assess for differences between two groups, student t test in SPSS (version 20) was used, and significance was defined as a value of $P < 0.05$. All figures are visualized by Prism 5.0. To assess the association between α-KL and other parameters, the Pearson correlation analyses were performed; and to obtain the predict value of serum α-KL, the area under the receiver operating characteristic (AUC-ROC) curve was conducted in SPSS.

Results

Basic information

Demographic and clinical characteristics of the subjects were summarized in **Table 1**. Age, pre-pregnancy BMI, and parity were comparable between two groups. However, the average gestation age of women with PE was lower than normal pregnancy (P=0.008), and neonates in PE group had lower birth weight (P=0.0002)

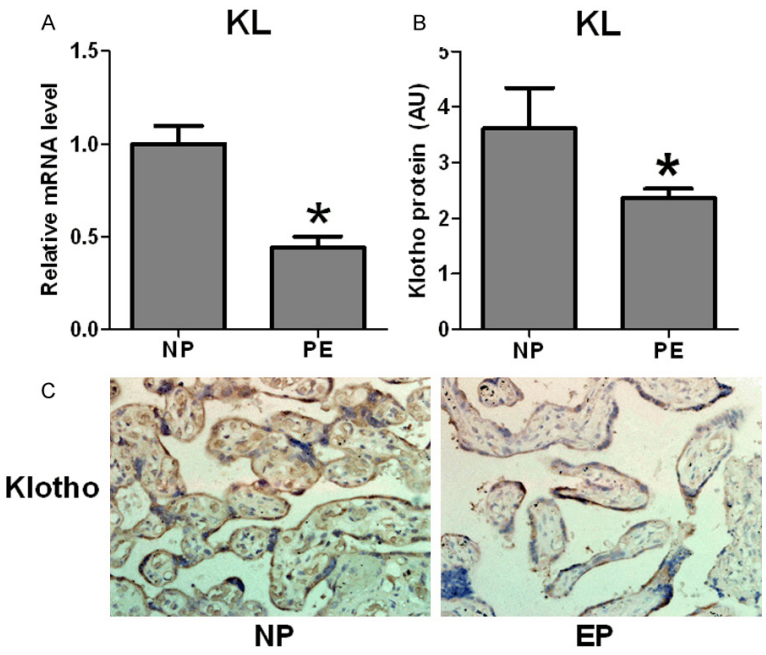

Figure 1. α-KL expression in placenta of PE and normal pregnant women. A: α-KL mRNA expression; B: Quantification of α-KL in immunohistochemistry; C: Immunohistochemistry of α-KL in placenta. NP: Normal pregnancy, PE: Preeclampsia. *: $p < 0.05$ when compared with NP; AU: Arbitrary unit.

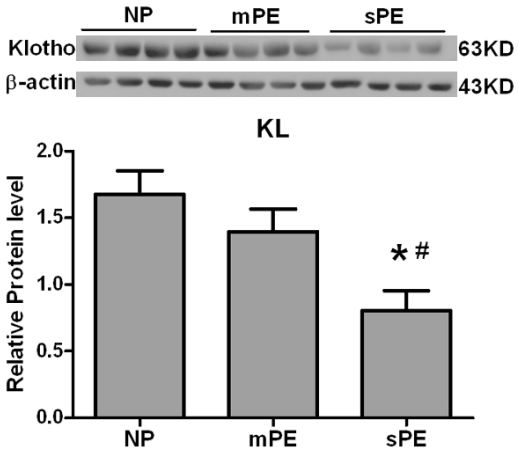

Figure 2. Western blot of α-KL expression in placenta of PE and normal pregnant women. NP: Normal pregnancy, PE: Preeclampsia. *: $p < 0.05$ when compared with NP.

and lower Apgar score (P=0.000) when compared with normal control.

The serum biochemical parameters were showed in **Table 2**. Levels of AL Band UA in maternal serum were significantly lower in PE than levels in normal pregnancy, but the SCr and urea concentration were higher in PE group than normal group.

Placental klotho mRNA and protein expression were lower in PE

Previous study showed that pregnant women had a higher plasma concentration of α-KL than non-pregnant women, and the plasma level of α-KL increased with the growth of gestational age [15]. We therefore first examined KL mRNA and protein expression in placenta. Real-time PCR indicated significant lower level of KL mRNA in placenta tissue of PE when compared with normal pregnant women (**Figure 1A**); and WB analysis showed that placental α-KL was detected as a major band with molecular weight of 63 kDa, and its expression level was also significant decreased in PE (**Figure 2**). IHC analysis showed that α-KL was expressed in cytotrophoblasts and the villi of connective tissue (**Figure 1B**, **1C**), which is in agreement with previous study [9].

Serum α-KL concentration was significantly lower in PE

KL protein exists in two forms: trans-membrane form and secreted form (α-KL). Trans-membrane KL is expressed primarily in renal tubular cells, while α-KL exists mainly in the blood, urine and cerebrospinal fluid. As for functions, trans-membrane KL influences more on fibroblast growth factor, and α-KL functions as a humoral factor that regulates activity of multiple glycoproteins on cell surface [3]. Recently, it was found that α-KL concentration was significantly higher in cord blood than that in non-pregnancy and neonates, indicating that α-KL might be a biomarker of PE [15]. Thus, we measured serum α-KL concentration and found that α-KL level in umbilical cord serum was markedly higher compared with that in maternal serum (**Figure 3A**), while umbilical cord serum α-KL concentration and maternal serum α-KL concentration had a positive correlation (r^2=0.145, p=0.0098) (**Figure 3B**). In PE group, α-KL levels were lower in both maternal and umbilical serum (**Figure 3A**).

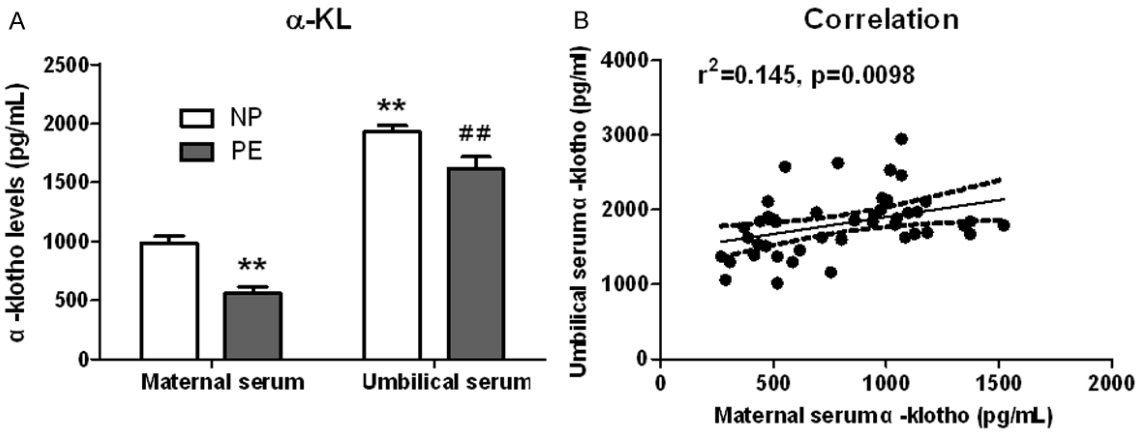

Figure 3. Serum α-KL level by ELISA. A: α-KL levels of both maternal and umbilical serum were lower in PE group; α-KL level in umbilical cord serum was markedly higher compared with that in maternal serum; B: Maternal and umbilical cord serum α-KL were positive correlated. NP: Normal pregnancy; PE: Preeclampsia; α-KL: α-klotho.

Serum α-KL concentration and fetal birth weight was positively correlated

Pregnant women with PE restrict the development of neonates, leading to low fetal birth weight. This is mainly due to the effect of PE on intrauterine growth restriction [16]. In order to investigate whether α-KL is related to low fetal birth weight caused by PE, Miranda et al. measured the median maternal plasma concentration of α-KL and found that it was lower in mothers with SGA neonates [9]. However, maternal pre-pregnancy BMI was not regarded as a controlled variable in their study. In the present study, we controlled maternal pre-pregnancy BMI and found that α-KL concentration (including maternal serum and umbilical cord serum) was positively correlated with fetal birth weight, both in normal group and in PE group. In addition, such correlation was more significant in maternal serum (r^2=0.384, p=0.0001 in all; r^2=0.32, p=0.0026 in NP and r^2=0.129, p=0.131 in PE) (**Figure 4A-C**) than in umbilical cord serum (**Figure 4D-F**).

Maternal serum α-KL concentration and SCr was negatively correlated

KL is predominantly expressed in the kidney and the concentration of α-KL decreases when kidney is injured [3]. The injury of glomerular endothelial cells and podocyte, which is closely related to KL, is one of the main causes of kidney dysfunction [17]. For pregnant women with PE, kidney dysfunction is one of the most common complications [2]. We hypothesized that kidney dysfunction is attributed to the decrease of α-KL in PE women. Our analyses revealed that maternal serum α-KL concentration was inversely correlated with SCr both in NC (r^2=0.192) and PE (r^2=0.233) (**Figure 5A-C**). Asimilar correlation between α-KL level and urea was observed in PE (r^2=0.163) but not in NC (r^2=0.003) (**Figure 5D-F**).

Serum α-KL may contribute to PE by modulating oxidative stress

It is known that increased oxidative stress exerts great impact on PE development and the occurrence of PE also exacerbates oxidative stress. Oxidative stress, which is influenced by α-KL through a variety of mechanisms, also affects fetal birth weight and maternal renal function [18, 19]. Our findings indicated higher oxidative (MDA concentration) and lower anti-oxidant (SOD concentration) capacity in PE group (**Figure 6**) when compared with normal pregnant women. Additionally, MDA was significantly higher in PE group (**Figure 7A**) and inversely correlated with maternal serum α-KL level (**Figure 7B**), and such correlation was weaker in PE group compared with normal group (r^2=0.13 in normal group; r^2=0.006 in PE). Our findings suggested that PE women may have the risk of lipid peroxidation and metabolic disturbance due to low expression of α-KL (**Figure 7C**, **7D**).

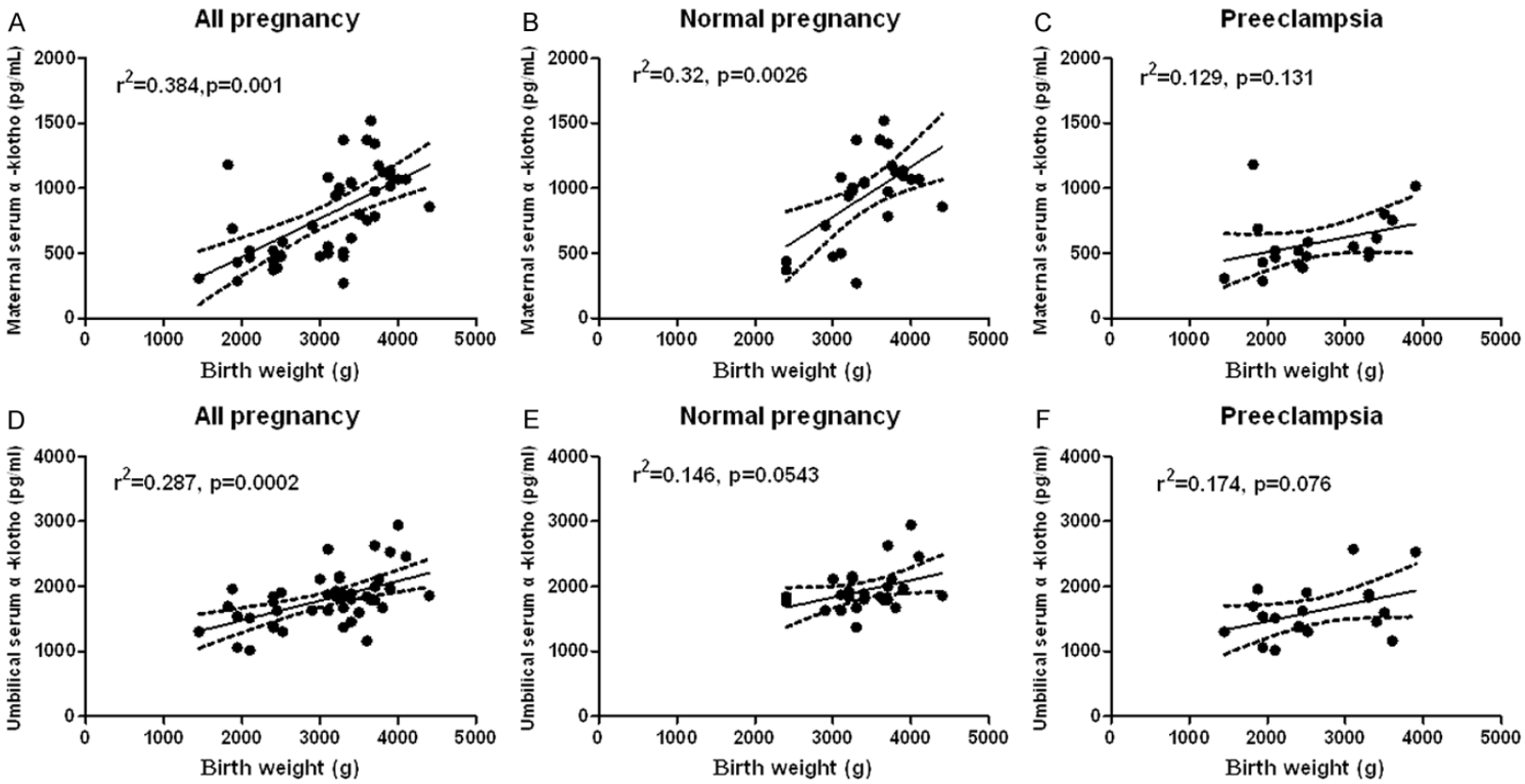

Figure 4. Correlation between α-KL and fetal birth weight. A-C: Maternal serum α-KL had positive association with fetal birth weight in normal pregnancy. D-F: Umbilical cord serum α-KL had positive association with fetal birth weight.

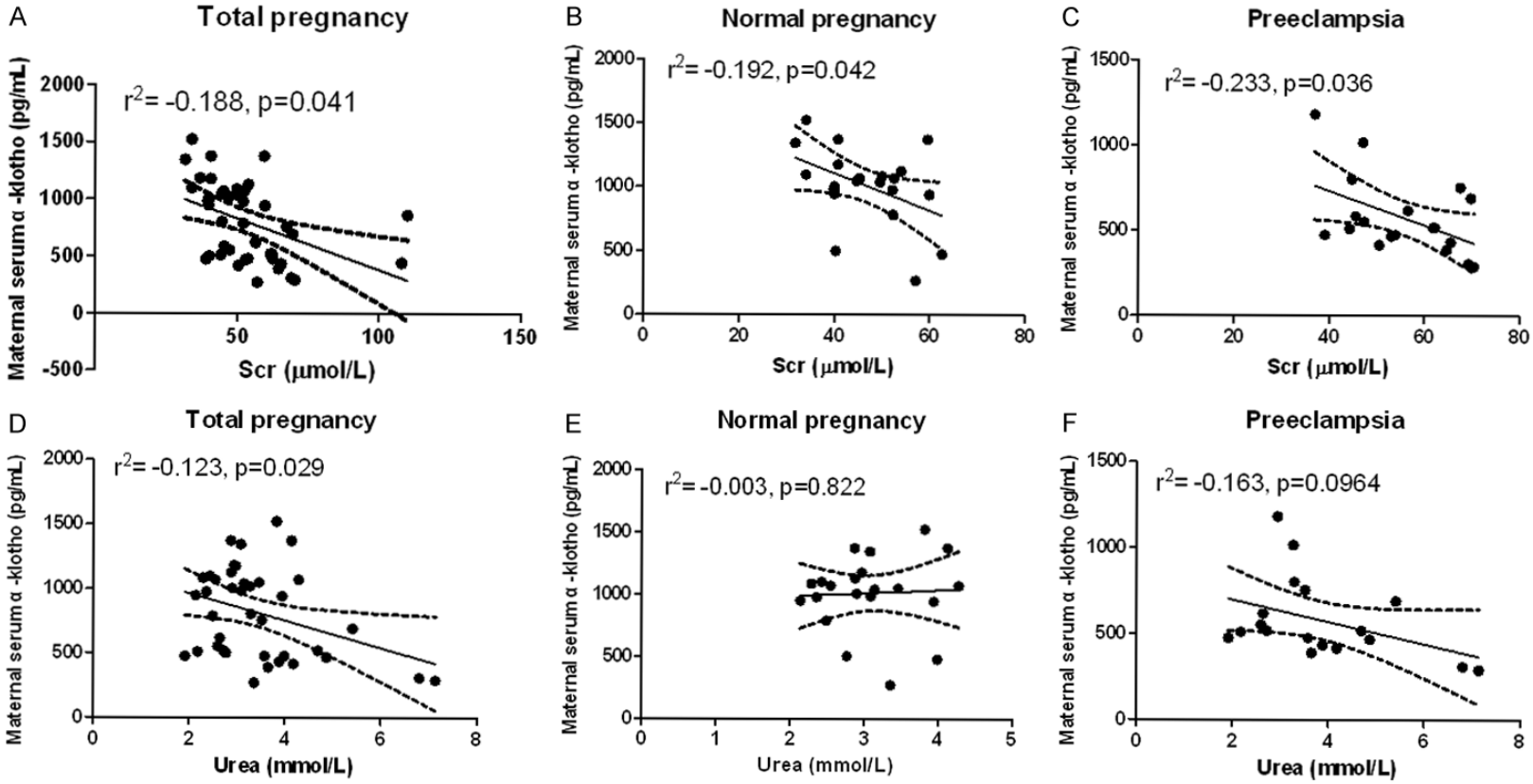

Figure 5. Correlation between serum α-KL concentration and SCr and urea. A-C: Maternal serum α-KL was inversely correlated with SCr both in NP and PE. D-F: The similar tendency remained in α-KL level and urea in PE but not in NP.

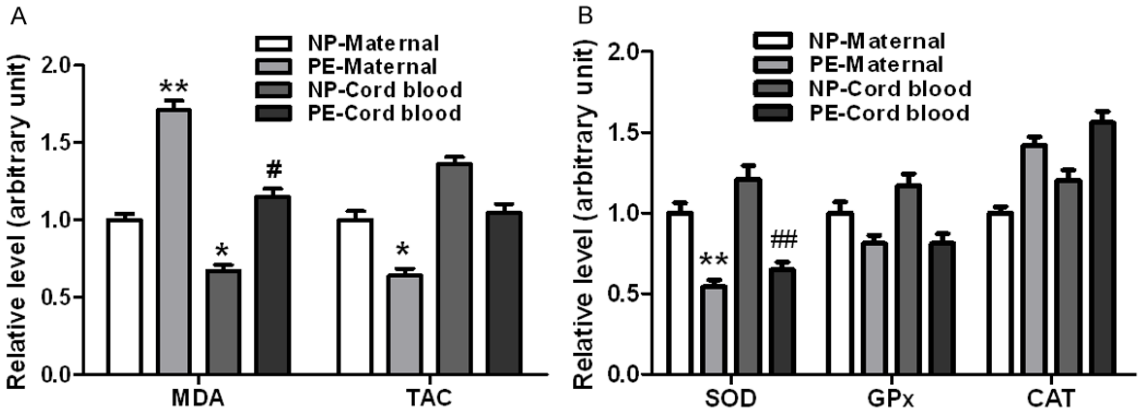

Figure 6. Oxidative stress and anti-oxidant capacity in PE. A: Maternal and umbilical cord MDA and TAC in PE and normal pregnancy. B: Anti-oxidant enzymes in PE and normal pregnancy. NP: Normal pregnancy; PE: Preeclampsia; MDA: Malondialdehyde; TAC: Total anti-oxidant capacity; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; CAT: Catalase.

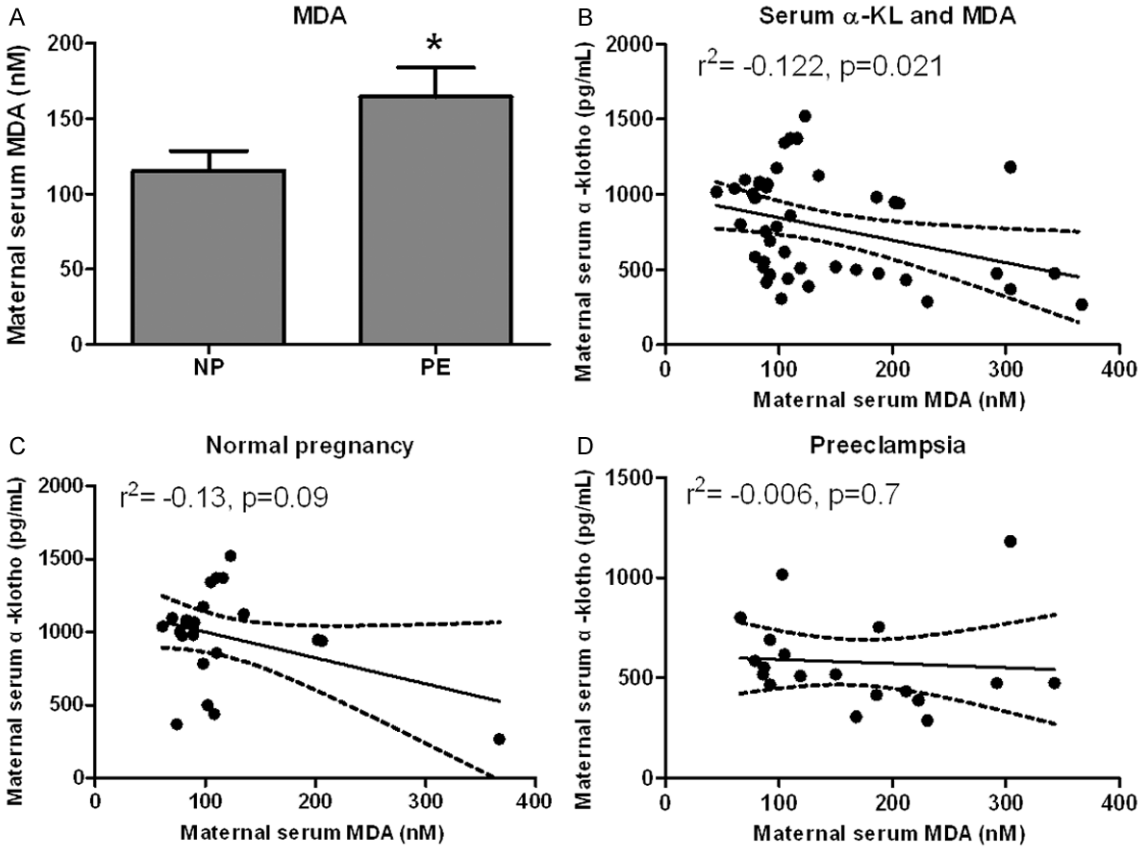

Figure 7. Association between MDA and α-KL. A: Maternal MDA was significantly higher in PE group; B: Maternal MDA inversely correlated with maternal serum α-KL; C, D: Such correlation weaken in PE group compared with normal group.

Serum α-KL could be a predictor of PE

Our results demonstrated that α-KL is linked with manifold dimensions of PE, we speculated that α-KL might be a novel predictor of PE. Therefore, we performed ROC curve analysis (**Figure 8**) and found that, with the optimal cut-off point 830 pg/ml, prediction of PE by serum

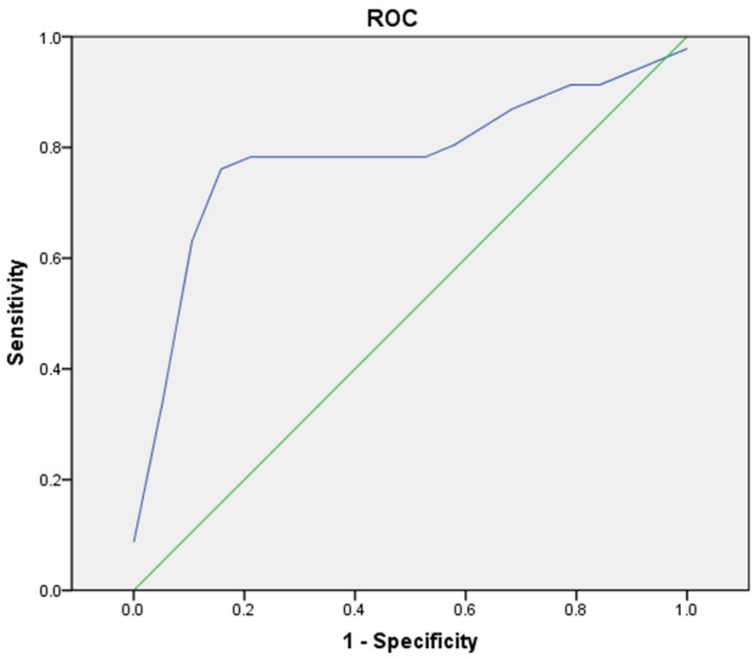

Figure 8. ROC curve analysis showed that serum α-KLmay predict PE with good accuracy. Optimal cut-off point 830 pg/ml, prediction of PE by serum α-KL reaches 89.5% of sensitivity (95% CI: 66.9-98.7) and 73.1% of specificity (95% CI: 52.2-88.4). And the model resulted in good discrimination with a ROC-AUC of 0.796, with 98.9% negative predict value (NPV), 20.3% positive predictive value (PPV) and Youden index of 0.63.

α-KL reached 89.5% of sensitivity (95% CI: 66.9-98.7) and 73.1% of specificity (95% CI: 52.2-88.4). And the model resulted in good discrimination with a ROC-AUC of 0.796, with 98.9% negative predict value (NPV).

Discussion

Our study measured α-KL expression in placenta and α-KL concentration in maternal and umbilical cord serum. We found that both α-KL mRNA and protein expression decreased significantly in PE women when compared with normal pregnant women; serum α-KL was higher during pregnancy than in non-pregnant state. In addition, women with PE showed lower serum α-KL level when compared with non-pregnant women. Maternal plasma α-KL concentration showed a similar tendency, as shown in a study conducted by Miranda et al. [9]. We also found a positive correlation between α-KL concentration and fetal birth weight. Previous studies have shown that when the maternal blood flow was re-established, there would be a rapid increase in tissue oxygenation [20]. This may explain why we observed significant association between α-KL and other physiological or biochemical parameters in maternal serum rather than umbilical cord serum.

PE had an adverse effect on both neonatal and maternal health, increasing diseases burden both mentally and economically. Numerous studies revealed that PE affected the development of neonates, leading to serious clinical outcomes such as small-for-gestational age, cardiovascular diseases, and childhood obesity. These complications were caused by the effect of PE on intrauterine growth restriction [16]. The clinical characteristics showed that PE patients had shorter gestational age and would deliver lower birth weight infants. We found that KL expression were markedly lower in PE women. Also, maternal and umbilical cord secreted α-KL were decreased significantly in PE women. Furthermore, serum α-KL and fetal birth weight was positively correlated, suggesting that α-KL may affect fetal growth restriction caused by PE. Researches with rats indicated that α-KL may be essential for survival after birth, but not for embryonic development [21]. Although there was few researches exploring the role of α-KL in infantile development, one possible explanation was that α-KL influenced fetal growth through the effect on placenta. In agreement with previous study, we found that α-KL was highly expressed in cytotrophoblasts and the villi of connective tissue. Additionally, expression level of KL was lower in PE placenta than in normal placenta. Redman *et al.* proposed that a powerful predisposing factor rather than the poor placentation was the cause of PE but was [22]. Additionally, Ohata *et al.* indicated that α-KL protein in infants was derived from placenta and placental syncytiotrophoblast was one of the major sources of the α-KL circulating abundantly in the fetus [15].

PE leads to maternal multi-organ dysfunction such as renal insufficiency, liver involvement,

and neurological complications [2]. Renal impairment is a type of severe morbidity associated with PE, and proteinuria is one of the vital diagnostic criteria of PE [23]. The development of renal impairment was derived from the injury of glomerular endothelial cells and podocyte, leading to the proteinuria [24]. Although it was suggested that the level of proteinuria should not guide management of PE [25], urinary proteome may contribute to better understanding of pathophysiology of PE, and new biomarkers may provide new strategies for the prevention or treatment of this syndrome. However, it was unrealistic to expect that a single biomarker could predict PE derived from pathways [26]. Clinical studies have suggested that α-KL was a potential reno-protective factor both in AKI and CKD, supporting by the reduction of renal α-KL expression in humans and several experimental animals [7]. Additionally, Hu et al. found that bloody α-KL expression level could be used as a detector of CKD [27]. Thus, α-KL may be a predictor of renal function in pregnancy with PE. In practice, SCr and UA are two biomarkers for renal function [28]. We found that serum α-KL was correlated with SCr as well as UA significantly and these results indicated that the detection of renal function could refer to α-KL concentration. Although the mechanism underlying how α-KL affects renal function is not fully understood, one plausible explanation is that increased renal α-KL expression may influence renal oxidative stress and lead to renal impairment [29].

The relationship between PE and oxidative stress was a vicious cycle where increased oxidative stress can induce PE and the occurrence of PE also exacerbates oxidative stress [30]. There was an early increase in oxidative stress in the placenta by the end of the first trimester before the clinical development of PE [30]. Some researches found that SGA infants had reduced antioxidant and increased prooxidant levels, and the insufficient uteroplacental led to placental hypoxia and increased oxidative stress. Sinceplacenta and uterine are places where developing fetus exchange gas and uptake nutrition from their mothers, fetal development is restricted when insufficient uterroplacental circulation occurs [18]. Accumulating evidence indicates that increasing α-KL is related to attenuation of oxidative stress and oxidative stress suppresses the transcription of α-KL [19]. The cause of oxidative stress was thought to be vascular endothelial cell injury, asearly onset PE was related with deficient conversion of the spiral arteries [31]. To be specific, the myometrial segments of the arteries were affected adversely [32]. However, PE was not only an endothelial disorder but also involves other components of the inflammatory network [33].

As a marker of oxidative stress, lipid peroxide formation was increased during pregnancy and PE [34]. Microvillous membrane lipid peroxide concentration can be quantitated as MDA, which doubled in pregnancy with PE in Cester's research [35]. The anti-oxidant enzymes such as SOD, catalase and GPx catalyze the reduction of the reactive oxygen intermediates. Specifically, SOD converts superoxide into hydrogen peroxide, which is translated into water by catalase or GPx [36]. Our results revealed that anti-oxidative capacity was greatly compromised in PE by the significantly higher MDA concentration and lower SOD concentration both in maternal serum and umbilical cord serum. Several studies revealed that α-KL deficiency increased oxidative stress, which could be rescued by administration of antioxidants [3]. Thus, we inferred that lower α-KL level in PE might be a plausible reason for no correlation between α-KL and oxidative stress in PE group.

It was suggested that α-KL could protect cells from oxidative stress by inducing the expression of antioxidant enzymes and reducing reactive oxygen species [37]. KL regulates insulin/IGF1 that is involved in the resistance to oxidative stress at the cellular and organismal levels in mammals [38]. This is via the negative regulation of forkhead box O (FOXO) byinsulin/IGF1 signaling [39]. FOXO binds to the promoters of genes encoding antioxidant enzymes directly if FOXOs were inactivated [40]. As we mentioned before, α-KL is related to uteroplacental circulation [15] and plays an important role in the two stages of PE, poor placentation as well as placental oxidative stress and inflammation [33]. With regard of the possibility that α-KL regulates oxidative stress and therefore contributes to PE, we explored the correlation between α-KL concentration and MDA. The obviously negative correlation between α-KL concentration and MDA in healthy pregnancy but not PE indicated that α-KL may regulate

anti-oxidative ability of pregnancy in certain way.

ROC curve analysis revealed that serum α-KL may predict PE with highaccuracy (AUC=0.796) in negative relationship (98.9% NPV). We proposed that monitoring α-KL concentration in maternal blood during pregnancy may helpto detect PE. However, the low positive predicted value may due to the low PE prevalence. But it was difficult to improve because the rate of incidence varies upon the study population but generally ranges from 3% to 7% of all pregnancies [23].

In conclusion, α-KL expression were significantly decreased in placenta of PE patients. Both maternal and umbilical cord serum α-KL were markedly lower in women with PE. α-KL regulate fetal birth weight as well as maternal renal function by modulating oxidative stress in PE (**Figure 7**). ROC analysis of 0.796 AUC suggested that α-KL might be a potential predictor of PE. However, current study has several limitations: first, this is a cross-sectional research, casual inference was limited. Secondly, the sample size was limited, further investi-gation with expanded sample size and perspective cohort design would give us greater power to dissect how α-KL contribute to PE development.

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Disclosure of conflict of interest

None.

Authors' contribution

All authors participated in developing the ideas presented in this manuscript, researching the literature, and writing parts of the text.

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