European Review for Medical and Pharmacological Sciences 2019; 23: 6026-6034

SHH expression in placental tissues and trophoblast cell oxidative stress injury during preeclampsia

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Abstract. – OBJECTIVE: To assess the association between Sonic hedgehog (SHH) protein expression levels on placental tissues and the presence of preeclampsia and to assess oxidative stress in cultured trophoblast cells from placentas of women with preeclampsia treated under different conditions.

PATIENTS AND METHODS: We enrolled 30 pregnant women with early-onset preeclampsia (<34 weeks), 30 with late-onset preeclampsia (≥34 weeks), and 60 healthy pregnant women with early or late gestational ages for two control groups. We used ELISA tests to measure individual serum SHH levels. Also, we used real-time PCR, and Western blotting to detect *Shh* transcript and protein levels in placental tissues, respectively, and immunohistochemistry to localize the protein in placental tissues.

We isolated trophoblast cells from placentas and cultured them in vitro under different conditions to compare oxidative stress injury and apoptosis rates between them.

RESULTS: The mean serum SHH levels in early and late preeclampsia groups were similar ($p>0.05$). Placentas of women with and without preeclampsia both expressed SHH protein mainly in syncytiotrophoblast cytoplasms. The mean placental SHH protein expressions in both women with early-onset and late-onset preeclampsia were significantly higher than that in the women of the late gestational age control group ($p<0.05$). The mean Shh mRNA abundances in early-onset and late-onset preeclampsia placentas were significantly higher than that in the late gestational age control placentas ($p<0.05$). The placental mitochondria oxidative stress injury was significantly higher in the preeclampsia than in the control group ($p<0.05$). The oxidative stress injury and apoptosis of trophoblast cells were higher in preeclampsia groups than in the late gestational age control group ($p<0.05$). Trophoblast cells treated with SHH serum had significantly lower oxidative stress than the same cells grown in the presence of preeclamptic serum ($p<0.05$).

CONCLUSIONS: The expression of Shh in placental tissues is associated with the oxidative stress mechanism during preeclampsia.

Key Words:
Preeclampsia, Sonic Hedgehog, Oxidative stress injury, Trophoblast.

Introduction

Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality. Its pathogenesis is incompletely understood and the proposed causal mechanisms include oxidative stress, abnormal trophoblastic infiltration, abnormal immune regulation, genetic causes, and nutritional disbalances. Oxidative stress is caused by the cumulative damage done by free radicals inadequately neutralized by antioxidants; that is, increased production of reactive oxidative species (ROS) or another oxidation imbalance causes a peroxidation state in the body. Normal pregnancy is accompanied by a general oxidative stress state, but the body's antioxidants remove the ROS free radicals and balance oxide formation and degradation processes maintaining homeostasis. Excessive ROS free radical production or decreased antioxidant production can lead to oxidative stress tissue damage. Many pregnancy complications, such as preeclampsia, pre-labor membrane rupture, and intrauterine growth restriction (IUGR) can be triggered by oxidative stress damage: Placental oxidative tissue damage is prominent in patients with preeclampsia[1]. Sharp AN et al found that stimuli including hypoxia and oxidative stress can cause placental apoptosis[2]. Preeclampsia, itself, also increases the apoptotic rate of placenta cells[3].

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The Hedgehog gene (Hedgehog, Hh) was discovered in 1980 when studying genetic mutations during Drosophila embryogenesis, it was named after the spiky projections of the Drosophila embryo back[4]. The Hh gene family and their signaling pathways were discovered later in vertebrates. The earliest discovered and best characterized gene in the Hh family is the Sonic hedgehog (*Shh*) gene, located at 7q36. *Shh* is a morphogen and mitogen important for embryonic development[5]. The SHH signaling pathway is involved in postnatal angiogenesis, inhibits apoptosis during ischemia, injury, and tumor formation[6], and has the potential to facilitate treatment of hypoxic-ischemic diseases[7]. The trophoblastic infiltration disorder caused by placental hypoxia and ischemia-reperfusion is an important placental change during preeclampsia, and we hypothesized that the SHH protein may be involved in the body's defense mechanism to fight placental oxidative stress injury.

To test our hypothesis, we examined the expression of Shh in placental tissues of women with preeclampsia and in those without it, cultured trophoblast cells *in vitro* to detect oxidative stress and apoptosis, and assessed the association between placental tissue *Shh* expression and the presence of preeclampsia.

Patients and Methods

Experimental Population and Grouping

Patient grouping and placental tissue collections

We obtained records from women who had prenatal checkups and who were hospitalized for cesarean section delivery between October 2012 and December 2013 at Fujian Provincial Maternity and Children's Hospital. We analyzed records from consecutive pregnant women scheduled for C-section, who had single pregnancies, lacked smoking or drinking habits, and had no special diets or lifestyles. In addition, the women had not yet entered labor and had had no premature membrane ruptures; the pregnancies were normal, except for the pre-eclampsia, and C-sections were successful and lacked postoperative complications.

The Ethics Committee of Fujian Provincial Maternity and Children's Hospital approved this study (FMCH2012-061). All of the patients signed informed consent forms. We divided the data from records into two groups of 30 women each presenting severe preeclampsia (one with early gestational age [<34 weeks] and one with late gestational age [≥34 weeks]) and two control groups of 30 women each without preeclampsia (early gestational age [<34 weeks] and late gestational age [≥34 weeks]). Since the women in the early-onset control group did not deliver their babies during the study period, we did not study their placental tissues.

The preeclampsia diagnoses were based on the standard guidelines from the Williams Obstetrics textbook (23rd Edition)[8]. We drew 3 ml of elbow venous blood from all the women and immediately injected them into glass test tubes without anticoagulant. After acclimatization at room temperature for 30 min, we extracted the serum by centrifugation and stored the samples in the -70°C freezer until experimental use within 2 months.

We collected placental tissues immediately after cesarean section delivery, taking 1.0 cm ×1.0 cm× 1.0 cm placental specimens from the umbilical cord root under aseptic conditions, and avoiding placental tissues with hemorrhage, infarction, or calcification. We stored tissues to be used for immunohistochemical localization in 4% paraformaldehyde; we rinsed tissues to be used for protein and mRNA measurements with cold physiological saline solution and placed them into sterilized 1.5-ml Eppendorf (EP, Hamburg, Germany) tubes, we then cooled down the EP tube immediately in liquid nitrogen, and stored the samples in a -70°C freezer for future usage.

Establishment of cultures from normal pregnancy placental tissues

We collected placental tissues under aseptic conditions in the cesarean section delivery room, we cut 3 pieces of 1.0 cm × 1.0 cm × 1.0 cm of placental tissues from the umbilical cord root immediately after the delivery avoiding placental tissues with hemorrhage, infarction, or calcifications. After rinsing in cold sterile saline, we placed the tissues in a phosphate-buffered saline (PBS) solution containing penicillin (10000 U/mL) and streptomycin (10 mg/mL) PBS, followed by immediate placement in an ice box, and then incubated them at 37˚C for 30 min. We then weighed each placental tissue specimen and divided each into 7 pieces (100 mg per piece), we cut each piece into 4 to 6 small pieces and placed each into a well of a 6-well culture plate for *in vitro* culture. We added sera (to a final concentration of 10%) from control pregnant women (control serum group), or from women with preeclampsia (preeclampsia serum group). We grew

the cells under these different conditions and used them to compare malondialdehyde (MDA) and super oxide dismutase (SOD) activity levels after incubation for 24 h at 37°C in a 5% CO_2 incubator (see procedures below).

Trophoblast cell line culture conditions

Human early pregnancy villous cell trophoblast cell line HPT-8 was purchased from the Central Laboratory of Xiangya School of Medicine, Central South University (China). We cultured the adherent cells in flasks containing DMEM/F12 medium with 10% bovine serum albumin in an incubator at 37°C and 5% CO_2. We trypsinized the cells at 80% confluence (growth rates averaged 3 to 4 days per generation), and resuspended them at a concentration of 5×10^4 cells/ml. We subcultured the live cells into 6-well culture plates and added different types of serum to them (see below) once confluence reached 80 to 90% and placed them back in the incubator for 24 h. We set up 4 different experimental conditions, growing cells in the presence or absence of serum and SHH for 24 h: (1) we grew cells in the presence of serum-free medium, (2) in DMEM/F12 medium containing 10% of healthy pregnant women serum, (3) in DMEM/F12 medium with 10% of preeclampsia patients' serum, or (4)in DMEM/F12 medium with 10% preeclampsia patients' serum (containing 500 ng/ml of SHH protein, according to our measurements)[9]. We used these cells to test MDA and SOD levels after growth under each condition.

Research Methods

Determination of serum SHH level

We used an enzyme-linked immunosorbent assay (ELISA kit, R&D, USA) and followed the manufacturer's instructions to measure SHH concentrations in patients' sera. After mixing the testing and standard samples, we added the biotinylated secondary antibody and enzyme-labelling reagent to the microtiter plates, we allowed reactions to proceed for 30 min at 37°C for 30 minutes, then we washed the plates 5 times, added the color developing solution, and developed the color at 37°C for 15 minutes before adding the stop solution. We then measured the 450 nm wavelength light absorbance optical density (OD) value of each well in a built-in microplate reader, and derived the regression formula of the standard curve according to the OD value and the concentration of the standard; finally, we calculated the concentration of each sample.

Immunohistochemical localization of SHH protein expression in placental tissues

After the placenta tissue dehydration and paraffin embedding, we sliced one sample from each placental group into 3-μm thick slices and adsorbed them onto adhesion slides for hot repair (66ºC for 60 min). We then dewaxed and hydrated the slides with graded ethanol, and immersed them into a sodium citrate solution under high temperature heating for 1-2 min for antigen repair. After cooling, we washed the slides with Tris-buffered saline (TBS). We used a 30% hydrogen peroxide solution to block endogenous peroxidase for 15 min, followed by incubation with sheep serum for 30 min at room temperature and binding with nonspecific antibody. We added the monoclonal mouse anti-human antibodies against SHH (Wuhan doctor DE Company, China) at a dilution of 1:100 to the slides and incubated them at 4ºC overnight. The next day, we added biotinylated sheep anti-mouse antibody (Beijing Zhong Shan Jin Qiao Company, China) at a dilution of 1:100 for 15 min at room temperature. After washing with TBS, we stained the specimens with diamino-benzidine and hematoxylin, and placed coverslips on them for microscopic observation. We used phosphate buffer solution instead of the primary antibody for the negative control sample.

Western blotting detection of SHH protein expression in placental tissue

We washed the placental tissues with PBS three times, and then with lymphocyte lysis buffer. We added extraction buffer to purify the proteins, and measured the concentrations using a BCA assay (Beijing ComWin Biotech, China). We solubilized cellular proteins in Laemmli buffer (Shanghai Biyuntian Bio-Technology, China) and denatured them by heating them at 95°C for 10 minutes. We ran the lysates in 10% SDS-PAGE and electro-blotted the proteins onto a polyvinylidene difluoride (PVDF) membrane. We blocked the membranes for one hour at room temperature in Tris-Buffered Saline and Tween-20 (TBS-T) containing 5% skimmed milk. Next, we incubated the membranes with the appropriate primary antibody (a rabbit monoclonal antibody against SHH, Abcam Company, UK) in TBS-T with 3% bovine serum albumine (BSA) overnight at 4°C. After washing the membranes with TBS-T, we incubated them with secondary antibodies (an anti-rabbit IgG antibody, Beijing

Dingguo Changsheng Biotechnology Company, China) for one hour at room temperature. Next, we washed the blots with TBS-T three times and used the BM Chemiluminescence system (Shanghai Jiehai Bio-Technology, China) for the detection. After exposure, we stained the membranes with Amido Black Staining Solution (New England Biolabs, Ipswich, MA, USA) to calculate variations in protein content among samples. We used the Basic Quantifier software (Shanghai Peiqing Science & Technology, China) to obtain a densitometric analysis of band intensities, and used β-actin as a loading control.

RT-PCR to detect the expression level of SHH mRNA in placental tissues

We extracted total RNA from frozen placenta samples using Trizol (Takara Biotechnology Dalian, China). We used a spectrophotometer to measure the light absorption values (A) and calculated the total RNA concentration and purity from the 260 nm and 280 nm absorption values. We ran a 1% agarose gel electrophoresis to verify the integrity of the purified RNA. We set up a reaction to reverse transcribe 2 μg of total RNA into cDNA, and adopted a real-time fluorescence quantification PCR method for quantitative determination. We obtained the full-length sequence of the target gene mRNA from GenBank and designed the primer sequences using Primer 5.0. We verified the specificity of the primers by performing a Blast analysis. All primers were synthesized by Beijing Dingguo Changsheng Biotechnology in China (Table I).

We followed the manufacturer's protocol to set up the RT-PCR reaction conditions and used a $2^{-\Delta\Delta CT}$ method to obtain a relative quantitative analysis.

Placenta and trophoblastic oxidative stress damage level detection

We determined placental tissue and trophoblastic mitochondria superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels using commercial kits (Shanghai Biyuntian Biotechnology, China) for mitochondrial extraction, SOD activity detection (total superoxide dismutase assay kit-WST), and MDA level detection, and we followed the manufacturer's instructions.

Trophoblast apoptosis level detection

We used an Annexin V-FITC apoptosis detection kit (Nanjing Kaiji Company, China) according to its instructions and a flow cytometer to obtain mean apoptosis levels for each group.

Statistical Analysis

We used the SPSS 18.0 software to statistically analyze the data we obtained. We presented measurement data as means ± standard deviations (x ± s), and used the *t*-test or the t'-test for comparisons between two groups. We used a one-way ANOVA analysis to calculate variance between groups. In addition, we used the Pearson correlation analysis to detect correlations between groups. We considered all $p<0.05$ as statistically significant.

Results

Comparison of General Conditions of Pregnant Women in Each Group to Confirm Preeclampsia Diagnoses

The systolic pressure (SP), diastolic pressure (DP), and mean arterial pressure (MAP) of the women in the early-onset and late-onset preeclampsia groups were higher than those in the control group ($p<0.05$). We found no significant differences in SP, DP and MAP between the early-onset and late-onset preeclampsia groups ($p>0.05$). See Table II.

Serum SHH Levels in Pregnant Women

The mean serum SHH levels in women with early-onset and late-onset preeclampsia were 40.35±30.41 ng/mL and 36.47±18.49 ng/mL, respectively. Early and late gestational age controls had similar mean levels at 31.09±12.13 ng/mL and 32.77±23.89 ng/mL, respectively ($p>0.05$). As shown in Figure 1, we found no significant differences between the early-onset and the late-onset preeclampsia groups, or between the early-onset

Table I. Primer Sequences.

	β-actin	SHH
Sense primer	5'GTTGCGTTACACCCTTTCTTGAC3'	5'GCGAGATGTCTGCTGCTAGTC3'
Antisense primer	5'CTCGGCCACATTGTG AACTTTG3'	5'GGATAAACTGCTTGTAGGCTAAAG3'
Probe sequence	5'ACTGCTGTCACCTTCACCGTTCCA3'	5'TCGTCTCCTCGCTGCTGGTATGCTCG3'

Table II. Test results of clinical indicators for pregnant women in each group (x±s).

Group	SP (mmHg)	DP (mmHg)	MAP (mmHg)
Early-onset preeclampsia group	161.02±11.51*	98.14±9.16*	119.47±3.70*
Late-onset preeclampsia group	153.45±8.52*	94.68±6.27*	118.22±5.56*
Early gestational age	113.84±6.55	72.79±7.23	83.75±4.72
Late gestational age	110.63±9.32	72.15±7.01	86.06±5.67

Note: Compared with the values on the same period in women of the control group, *p<0.05
SP, systolic blood pressure; DB, diastolic blood pressure; MAP, mean arterial pressure.

and the late-onset control groups in terms of serum SHH levels (both p>0.05).

Comparison of Levels of Placental SHH Expression in Each Group

Localization of SHH protein expression in placentas

The immunohistochemistry results showed SHH expression in normal and preeclamptic placentas, mainly in the cytoplasm of syncytiotrophoblasts. The positive staining showed brownish yellow particles in the tissue, next to laryngeal carcinoma tissue as a positive control, as shown in Figure 2.

SHH protein expression levels in each placental tissue group

Our Western blot results indicate that SHH was expressed in normal and preeclamptic placentas (Figure 3). We normalized the density of the SHH bands in relation to those of β-actin. The density values for placentas with early-onset and late-onset preeclamp-

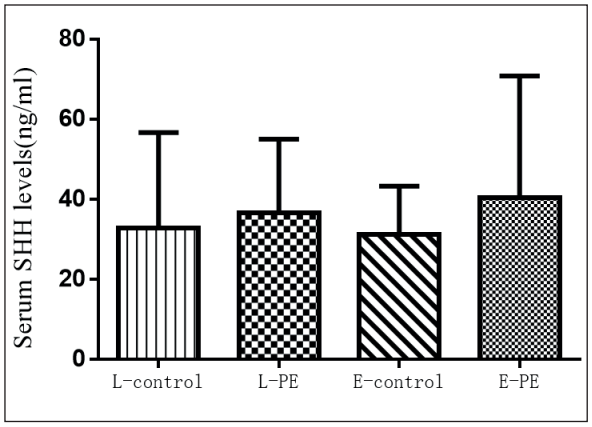

Figure 1. Serum SHH levels of pregnant women in each group (L-control are control women with late gestational age, L-PE are women with late-onset preeclampsia, E-control are control women with early gestational age, E-PE are women with early-onset preeclampsia).

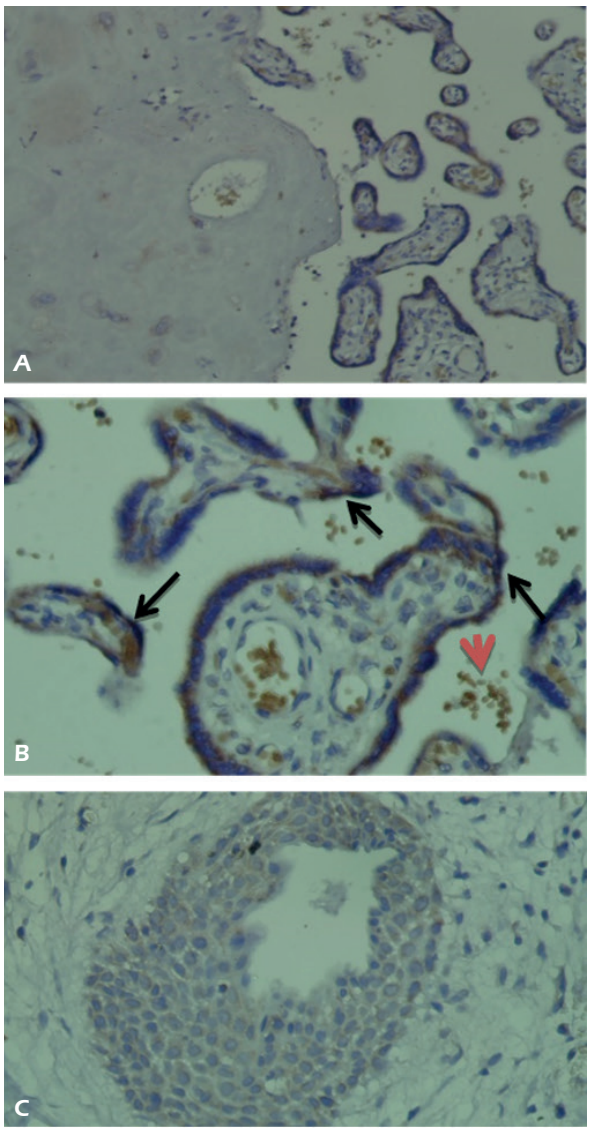

Figure 2. Expression distribution of SHH in representative placental tissue. ***A***, Expression of SHH in placenta (40×), see positive brown-stained syncytiotrophoblast layer and negative expression of the cytotrophoblast layer. ***B***, Black arrow indicates SHH positive expression in placental syncytiotrophoblast layer (100×), red arrow indicates red blood cells in the blood vessels and villus gaps. ***C***, Deep staining of keratinized beads in laryngeal carcinoma tissue (40×), SHH positive expression.

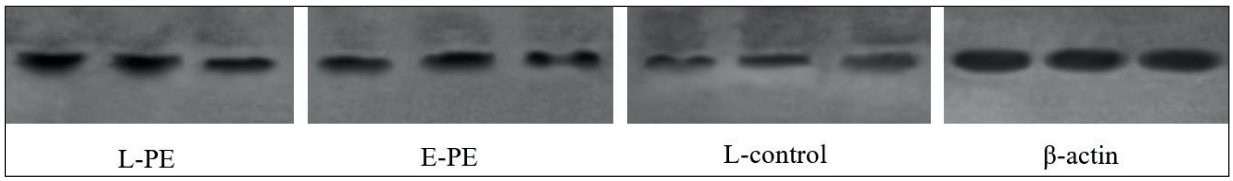

Figure 3. Expression level of SHH protein in placental tissues according to Western blot results.

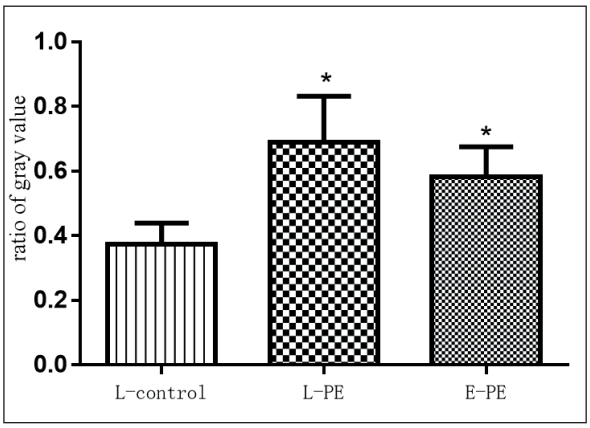

Figure 4. SHH protein expression level in placenta of each group (L-control refers to women in the late gestational age control group, L-PE refers to women with late-onset preeclampsia, E-PE refers to women with early-onset preeclampsia. Note: compared with late gestational age control group, *p<0.05).

sia were 0.58±0.09 and 0.69±0.14, respectively. The density value for the control group was 0.37±0.07. We found significant differences between the early-onset preeclampsia group and the control group, and between the late-onset preeclampsia group and the control group (p<0.05). We found no significant differences between the values of the early-onset preeclampsia group and those of the late-onset preeclampsia group (p>0.05), see Figure 4.

SHH mRNA expression levels in different placental groups

Table III shows the relative *Shh* mRNA contents in placentas. The relative Shh mRNA content in placentas of early-onset and late-onset preeclampsia groups were 1.86±0.52 and 1.79±0.24, respectively; while that of the control group was 0.90±0.087. We found significant differences between the values for the early-onset preeclampsia group and the control group, and between that of the late-onset preeclampsia group and the control group (p<0.05). The values for SHH mRNA contents between the early onset preeclampsia group and the late-onset preeclampsia group were similar (p>0.05), see Figure 5.

Comparison of MDA and SOD activity in placenta tissues

We found the MDA levels were higher and the SOD activity levels were lower in the cells grown in the presence of preeclampsia serum than in cells grown in the presence of control serum, and the difference was statistically significant (p<0.05), as shown in Table IV.

Comparison of Oxidative Stress Damage and Apoptosis in Trophoblast Cell Lines of Each Group

The levels of MDA and apoptosis in the trophoblast cell lines grown in the presence of preeclamptic serum were higher, and the SOD activity level was lower than those in trophoblastic cells grown in the presence of control sera, and the differences were statistically significant (p<0.05). The trophoblasts grown in the presence of serum containing SHH demonstrated lower MDA and apoptosis levels and higher

Table III. Comparison of CT value and relative content of SHH mRNA in each group.

Group	CT Value	$2^{-\Delta\Delta}$CT
Early-onset preeclampsia group	27.68±0.49	1.86±0.52*
Late-onset preeclampsia group	27.31±0.36	1.79±0.24*
Late gestational age	28.78±0.62	0.90±0.09
β-actin	16.39±3.17	—

Note: *p < 0.05 compared with late gestational age, "—" means no such item

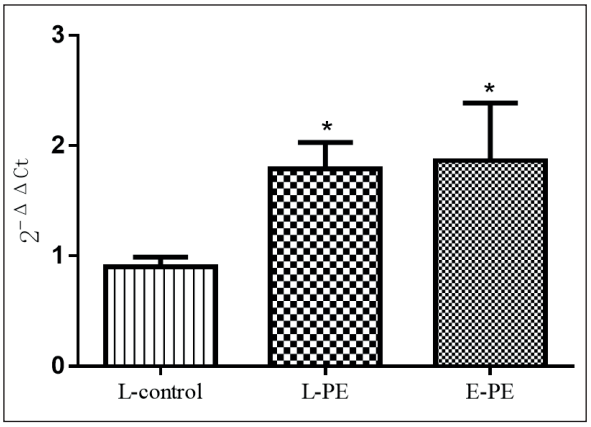

Figure 5. SHH mRNA expression level in placentas of each group (L-control refers to women in the late gestational age control group, L-PE refers to women with late-onset preeclampsia, E-PE refers to women with early-onset preeclampsia. Note: compared with late gestational age control group, *p<0.05)

SOD activity levels (p<0.05) than the trophoblasts grown in the presence of preeclamptic serum (Table V).

Discussion

Preeclampsia, a disease unique to pregnancy, is considered the leading cause of maternal and perinatal morbidity and mortality [10]. Unfortunately, its pathogenesis remains unclear, but placental ischemia and hypoxia have been proposed as causal events[11]. During a normal pregnancy, the body's oxidative and anti-oxidative effects remain relatively balanced. Superoxide dismutase (SOD) acts as one of the main oxygen radical scavenging enzymes in the body, and it can catalyze the reaction in which excessive peroxides are turned into hydrogen peroxide and oxygen. Endogenous or exogenous noxious stimuli can break this balance, causing a large amount of reactive oxygen species (ROS) to form and exceed the ability of the antioxidant system to clear them. Free radicals peroxidize lipids producing malondialdehyde (MDA) as a final product. MDA is toxic to cells, and it is marker of oxidative stress. In the middle and late terms of pregnancy, if the placental perfusion is reduced, the body compensates by re-perfusing it, resulting in a large amount of ROS free radicals[12]. The accumulation of ROS triggers cell damage, decreased intracellular SOD synthesis, and decreased SOD activity. Cellular ROS molecules that do not get neutralized on time cause damage to the circulation and lead to the oxidative stress associated with preeclampsia. We found that the placenta and trophoblast cells grown in media containing serum from women with preeclampsia had increased MDA levels and decreased SOD activity levels than those grown in the presence of serum from women with normal pregnancies, this suggests that oxidative stress may damage the placenta of patients with preeclampsia[13].

The SHH signaling pathway plays a role in postnatal angiogenesis and has anti-apoptotic effects during ischemia, injury, and tumor formation. Studies have reported that cell proliferation and upregulation of SHH signaling pathways occur in patients with myocardial[14], testicular[15], liver[16], kidney[17], brain[18], and other tissue damages,

Table IV. MDA and SOD activity levels of placental tissues in each group ($\bar{x}$± s).

Group	MDA (nmol/mg protein)	SOD (U/mg protein)
Preeclampsia serum group	55.85±6.25*	77.52±9.92*
Control serum group	50.20±7.11	92.45±7.17

Note: "*" indicates p<0.05 compared with the control serum group p<0.05

Table V. MDA, SOD activity, and apoptosis levels of trophoblasts in each group ($\bar{x}$± s).

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	Early apoptotic rate (%)	Late apoptotic rate (%)
Preeclampsia serum	216.75±8.09[a]	39.23±4.74[a]	37.11±5.67[a]	2.78±0.61[a]
SHH treatment	177.05±10.82[b]	57.11±7.67[b]	9.14±1.35[b]	0.79±0.14[b]
Control serum	156.07±9.05	54.42±4.56	0.74±0.13	1.02±0.29

Note: compared with the control group 2, [a]p<0.05; compared with preeclampsia group, [b]p<0.05.

highlighting the importance of SHH during tissue damage repair. We found that MDA levels are decreased and SOD activity levels are increased in trophoblast cells grown in the presence of SHH serum in their media, when compared to the levels in cells grown in the presence of preeclamptic serum without SHH. In addition, placental tissues in preeclampsia patients had higher SHH protein and mRNA expression levels than those in healthy pregnancy placentas, suggesting that placental oxidative stress injury in preeclampsia patients leads to compensatory SHH expression, which plays a role in tissue damage repair.

We also examined the level of trophoblast apoptosis in medium with preeclamptic serum with SHH, and found that the level of trophoblast apoptosis was lower than that in medium from women with preeclamptic serum without added SHH, indicating that SHH may prevent trophoblast apoptosis during the development of preeclampsia. *In vitro* model studies on the therapeutic effect of exogenous SHH have found that exogenous SHH has anti-oxidation and anti-apoptotic effects[19,20]. Dai et al[19] used H_20_2 to simulate oxidative stress in neuronal cells and found that after exogenous SHH addition, the cortical neurons produce less ROS and more antioxidant SOD levels, and that they had higher glutathione peroxidase (GSH-PX) activities and less decreased lipid peroxidation MDA levels than cells without added SHH. The SHH signaling pathway prevents apoptosis in a variety of tissues. The molecular level mechanism is suspected to involve the SHH signaling pathway linked to the Bcl-2 promoter region via Gli-1 and facilitating inhibition of the apoptotic Caspase (induced by death- causing FAS ligands) by Bcl-2 and insulin-like growth factor-binding protein (IGFBP)[20].

According to our immunohistochemical localization results, SHH is expressed in healthy and preeclampsia placentas, distributed mainly throughout the syncytiotrophoblastic cytoplasms. This may help explain why the apoptosis rates of syncytiotrophoblast cells are much higher than those of cytotrophoblast cells[2]. In addition, as the trophoblast cells of the placental villous cells proliferate, some of the syncytiotrophoblast cells undergo apoptosis to stabilize the functional integrity of the placental tissue. During preeclampsia, the placenta becomes hypoxic, and trophoblast cells proliferate rapidly, so the rate of integration into the syncytiotrophoblast layer increases accordingly. To maintain a balanced cell number, the apoptotic rate of syncytiotrophoblast cells is further increased, which may lead to a compensatory increase in the anti-apoptotic effect of SHH.

Conclusions

Our results show that changes in SHH expression in placental tissues may be associated with the oxidative stress mechanisms during preeclampsia. Our study suggests a new approach for clinical prevention and treatment of preeclampsia. However, our results are only preliminary and further exploration is needed to uncover the whole SHH mechanism in oxidative stress injury during the development of preeclampsia.

Acknowledgments

This work was supported by the Key Clinical Specialty Discipline Construction of Fujian, P.R.C (2015, number 593), by the Fujian Medical Innovation Subject (2017-CX-11), and the Fujian Science and Technology Leading Project (2018Y0005).

Funding

Key Clinical Specialty Discipline Construction of Fujian, P.R.C ([2015] no. 593), Fujian Medical Innovation Subject (2017-CX-11) and Fujian Science and Technology Leading Project (2018Y0005).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LQP, YJY, and XQ conceived and designed the study; LQP and XQ carried out the experiments; LQP, YJY, and XQ analyzed the data and wrote the paper; and YJY reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of Fujian Provincial Maternity and Children's Hospital approved all protocols (FMCH2012-061).

Patient consent for publication

We obtained informed consent forms for all of the patients recruited.

Competing interests

The authors declare that they have no competing interests.

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