

Lost OTR Expression and HIF-1 α Self-protection in Myometrium of Intractable Postpartum Hemorrhage

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Abstract

Objective: In intractable postpartum hemorrhage (IPPH), uterotonic lost efficacy in myometrium contraction formation. While the key target in uterotonic, OTR (oxytocin receptor) and internal environment in myometrium might have some changes when IPPH.

Methods: Three groups of myometrial tissue at different contraction states were collected: (1) term not at labour (TNL) group (10 cases); (2) term at labour (TAL) group (10 cases); (3) intractable postpartum hemorrhage (IPPH) group (10 cases). Expressions of OTR and internal environment markers (ASIC1a, HIF1 α , GLUT, FATP and apoptosis) were compared in 3 groups by Immunoreactivity Scores method.

Results: The expression of OTR in myometrium in IPPH group was 100% negative, compared to 30%, 20% in TNL and TAL group ($p < 0.05$). 70% positive HIF-1 α expression in TNL group increased to 80% in TAL group, but decreased to 20% in IPPH group ($P < 0.05$). While the positive expressions of ASIC1a, FATP and GLUT in three groups all had no significant difference. But the apoptosis related markers Bax, caspase-3 and caspase-9 positive expressions in IPPH were significantly all decreased (10%, 10%, 0%) compared to TNL and TAL group, compared to a high positive expression of Bcl-2 in 3 groups.

Conclusion: OTR expression and HIF-1 α self-protection might be lost in myometrium of IPPH, which could lead to uterotonic lost efficacy and conservative treatment failure.

Introduction

Intractable postpartum hemorrhage (IPPH), which could not be effective hemostasis by conservative treatments and exceed to more than 1500ml bleeding, always lead to severe coagulation dysfunction (DIC), MODS or even maternal near miss [1]. The pathogenesis and mechanism in intractable postpartum hemorrhage (IPPH), has always been the difficulty in clinic.

When dealing with postpartum hemorrhage, uterotonic, such as oxytocin, ergot alkaloid and hemabate are always the first choice which have been proved to have great effects on normal tocolytic fatigue [2]. But in IPPH (intractable postpartum hemorrhage), these drugs seem to lost their efficacy, to which myometrium seem to lose sensitivity. In this situation, more surgical interventions or even hysterectomy may be needed to save women's life in obstetric clinic [3]

OTR (oxytocin receptor), the key target in uterine contraction proteins (UAPS), always play an important role in uterotonic effect. In myometrium, activation of OTR can increase the opening of Ca²⁺ channels on cell membrane and sarcoplasmic reticulum (SR), and subsequent phosphorylate myosin light chains by MLCK to bring cross-bridge cycling and generation of contraction force, which was considered the canonical stimulation pathway by OTR [4]. At IPPH, the myometrium lost sensitivity to uterotonic, which could be speculated that OTR or its related downstream expressions might present obstacles.

Many studies have shown that uterine contraction mediated by OTR could be affected by internal environment, such as PH, hypoxic environment, energy metabolism, etc[5]. When IPPH (intractable postpartum hemorrhage) presents, the whole body is in a catastrophic hemorrhagic shock state, and the internal environment in cells might be also in a severe acidosis, hypoxia, metabolic stability disorder or apoptosis state. ASIC1a, widely expressed in the central and peripheral nervous systems, is an acid-sensing ion channel in cell membrane. ASIC1a is the important key receptor in acid environment when PH change, which plays an important role in the transmission of information[6]. While HIF1 α (Hypoxia-inducible factor-1 α) is the core factor that changes when the body responds to hypoxic stress, and is the key to regulating the oxygen balance at cellular level[7]. In cardiac and skeletal muscle disease, GLUT (Glucose transporter) and FATP (Fatty acid transporter), which could reflect glucose and fat metabolism level, have been proved to play important role in related pathogenesis researchs[8]. So when in IPPH, possible acidosis, hypoxia and metabolic stability disorder in myometrium could be reflected in ASIC1a, HIF1 α , GLUT and FATP expression changes, respectively, which might affect OTR expression in that situation.

So in view of IPPH state, which is lack of reactivity with uterotonic, we speculated that OTR expression, and its related internal environment factors (acidosis, hypoxia, metabolic stability disorder or apoptosis) might have some abnormalities or obstacles in myometrium cells. Therefore, in this study, we will compare their expression differences in IPPH with other contraction states in myometrium, in order to explore the pathogenesis and mechanism of IPPH, which will provide the new target and idea for future prevention and treatment of IPPH.

Materials And Methods

1.1 Different myometrium contraction groups

Women with singleton pregnancy, full-term (gestational age 38+0 weeks to 40+5 weeks) were included in this research. But due to the very low incidence of hysterectomy because of intractable postpartum hemorrhage, we selected paraffin pathological specimens for uterus in the last 10 years from Guangzhou Women and Children's Medical Center, Zengcheng Maternal and Child Health Hospital and the Third Affiliated Hospital of Guangzhou Medical University.

Three groups of myometrial tissue at different contraction states were collected: (1) term not at labour (TNL) group (10 cases): elective cesarean section due to abnormal fetal position or umbilical cord factor and with no postpartum uterine atony; (2) term at labour (TAL) group (10 cases): at labor with emergent cesarean section with no postpartum uterine atony or hemorrhage; (3) intractable postpartum hemorrhage (IPPH) group (10 cases): patients experienced labor but finally need hysterectomy due to ineffective conservative treatment for intractable postpartum hemorrhage.

The sample collections in 3 groups the study were approved by the Ethics Committee of Guangzhou Women and Children Medical Center, and participants signed informed consent. Ethics number: No.2018041701.

1.2 Sampling and storage

In the first 2 groups, myometrial tissue measuring 1 cm x 1 cm x 1 cm were collected from the upper border of the uterus right after placenta delivery. After washing with 0.9% normal saline, it was quickly stored in liquid nitrogen for further treatment. A section of each of the biopsies collected were made into paraffin blocks, and, the remaining myometrial tissue was stored in a refrigerator at -80°C for subsequent experiments.

1.3 Immunohistochemical staining

Indirect avidin-biotin-peroxidase complex method was used to detect immunoreactivity of those markers. Deparaffinated, rehydrated sections were incubated with 0.3% H₂O₂ to block endogenous peroxidase activity; then antigen retrieval was performed by boiling sections in Tris-EDTA buffer. The sections were rinsed in PBS, blocked with 10% goat serum, and then separately incubated with rabbit anti rabbit anti-OTR polyclonal antibody (1:100, Abcam Company), rabbit anti- HIF1a polyclonal antibody (1:100, Abcam Company), rabbit anti-ASIC1a polyclonal antibody (1:150, Thermofisher Company), rabbit anti-FATP-1 polyclonal antibody (1:100, Abcam Company), rabbit anti-FATP-4 polyclonal antibody (1:100, Abcam Company), mouse anti-GLUT2 monoclonal antibody(1:100, Abcam Company) , rabbit anti-GLUT4 polyclonal antibody(1:100, Abcam Company) ,rabbit anti-Bcl-2 polyclonal antibody(1:100, Abcam Company), rabbit anti-Bax polyclonal antibody(1:100, Abcam Company),rabbit anti-Caspase-3 polyclonal antibody(1:100, Abcam Company) and rabbit anti-Caspase-9 monoclonal antibody(1:100, Abcam Company).

After washing in PBS, the slides were incubated with a biotinylated secondary antibody (biotinylated goat anti-rabbit IgG secondary antibody). The sections were rinsed once more in PBS , incubated with ABC complex and visualized using DAB chromogenic agent. Counterstaining was done with 10% Mayer's Haematoxylin then washed in cold water and mounted with glycerol-gelatine.

1.4 Imaging analysis

The Leica DM4B light microscope was used for evaluation, and each section was photographed by Leica digital camera. 5 high magnification visions (400 times) were randomly selected in each immunohistochemical section, 100 trophoblast cells were observed in a high magnification visual field. According to the proportion of positive cells and staining intensity of positive cells, we made a semi quantitative score grade. The staining intensity level scoring was described as: non staining for 0 point, faint staining for 1 point, moderate staining for 2 points and strong staining for 3 points. Positive rate of staining cells scoring was:<5% for 0 point, 5%~25% for 1 point, 26%~50% for 2 points, 51% ~75% for 3 points and >75% for 4 points (Table 1). Two scores are added to total score grade: score 0-1 for negative(-), score 1-3 for weak positive(1+), score 3-4 for moderate positive(2+) and score 5-7 for strong positive(3+).The real positive expression was considered as total score ≥ 3 , otherwise the expression was negative.

1.5 Statistical analysis

The statistical analysis was performed by SPSS software (SPSS Inc., version 22). The immunohistochemistry results were compared between groups using the Fisher's exact test. Spearman test was used for correlation analysis. As for the comparison between groups, a P value less than 0.05 was considered as statistically significant.

Results

1. Clinical characteristics of study participants

A total of 30 pregnant women were enrolled in this study. The demographic and obstetrical characteristics of all participants were shown in Table 2. All groups were similar in maternal age, body mass index ($P > 0.05$). IPPH (Intractable postpartum hemorrhage) group had more postpartum bleeding, together with more duration of labor and neonatal weight $P < 0.01$.

2. Expressions of OTR in IPPH and other contraction groups in myometrium

Immunohistochemical results showed OTR was expressed in cytomembrane. The expression of OTR on myometrium in TNL group and TAL group showed moderate positive 40% and 40%, and strong positive 30% and 40% expressions, respectively, (Table 3). However, there was no moderate or strong positive expressions in IPPH group, which was 90% negative and 10% weak expression, indicating that the expression of OTR was significantly lost in IPPH, which was significantly different from the previous two groups ($P < 0.05$) (Figure 1).

3. Expression of acidosis, hypoxia factors in myometrium groups

As an indicator of hypoxia, HIF-1 α expression in the TNL group, with a moderate positive of 70%, and in the TAL group, with a moderate positive of 40% and a strong positive of 40% (Figure 2A). However, in IPPH group, the moderate positive decreased to 20% and the strong positive was 0% (Table 3), which was significantly lower than the TNL and TAL groups ($P < 0.05$). The moderate positive expressions of ASIC1a in the three groups was TNL 50%, TAL 60%, and IPPH 40%, while the strong positive expression was TNL 20%, TAL 10%, and IPPH 20% (Figure 2B). There was no significant difference in the positive expression rate between the three groups ($P = 0.910$). (Table 3)

4. Expressions of lipid and glucose metabolism factors in myometrium groups

FATP1 was positively expressed with a certain degree on the myometrium membrane in three groups, in which the moderate positive was 50% (TNL), 50% (TAL), 40% (IPPH) (Figure 3A), with no significant statistical difference ($P = 0.948$). However, FATP4 had less positive expression, with the moderate positive levels of 10% in all three groups (Figure 3B), also with no statistical difference in expression ($P = 0.804$) (Table 4).

The positive expression rates of GLUT2 on the myometrium membrane were high(Figure 3C), with moderate positive 60%(TNL), 50%(TAL), 50%(IPPH),and strong positive 10%(TNL) ,20%(TAL), and 20% (IPPH), respectively, with no significant statistical difference($P=0.833$).GLUT4 expression changes were similar to GLUT2(Figure 3D), with 60%(TNL) , 70%(TAL) , and 70%(IPPH) positive,respectively, also with no statistically significant difference ($P=0.935$)(Table 4).

5. Expression of apoptosis factors in myometrium groups

In classic apoptosis markers, Bax expression positive rates in TNL and TAL group were 90%, 60% compared to 10% in IPPH group (Figure 4A), with significant statistical difference($P<0.01$). The expressions of caspase-3 and caspase-9 also showed the same trend as Bax. The overall positive expression rates were caspase-3 (TNL 70%, TAL 50%, IPPH 10%) and caspase-9 (TNL 60%, TAL 50%, IPPH 0%) (Figure 4C and 4D), in which expression was significantly all decreased in IPPH group.However, the expression of Bcl-2 in the three groups all presented a high positive expression rate (TNL 70%, TAL 70%, IPPH 80%)(Figure 4B), and with no significant statistical difference ($P=0.899$)(Table 5).

Discussion

Intractable postpartum hemorrhage(IPPH) ,in which a large amount of blood loss will occur in a short period of time,is a serious complication of perinatal period. Quickly and effectively compressing myometrium to stop bleeding is very important in IPPH rescue time[9]. The effective speed of uterotonic to form myometrium contractile force, as the first step in the classical route of postpartum hemorrhage treatment , is always of great concern in hemostasis in which OTR is an important target for regulating myometrium contractility[10]. However, the reactivity of myometrium to uterotonic show declined sharply in IPPH mostly just as in prolonged labor[11] but studies on the regulation of myometrium contractility are still lacking.

The expressions of OTR(oxytocin receptor) have dynamic changes in pregnancy and childbirth in human, which may play an important role in physiological regulation of pregnancy and childbirth. Fuchs AR et al. Found that OTR expression was low in early pregnancy, but increased to 12-fold by 37-41 weeks, and maximally expressed after delivery[12]. Arthur P. et al. also documented gestation- and labour- dependent changes in expression of OTR and related genes in rat uterus[13]. In our research, OTR had strong expressions in TNL group and TAL group as reported, but in the IPPH group, lost OTR expressions were shown, which could reveal the etiology of the ineffectiveness of uterotonic agents. And this also could tell us continuous application of uterotonic agents might be useless mostly when meeting intractable postpartum hemorrhage, thus timely surgical interventions or even hysterectomy were needed to hemostasis for saving lives.

OTR belongs to the G protein-coupled receptor superfamily, and its expression changes are regulated by receptor desensitization and local changes in oxytocin concentration[14].In desensitization, membrane receptors are internalized by classical clathrin-mediated pathways, and then are targeted for recycling to cell membranes or degradation by lysosomes[15]. Talati,C found myometrium remain more responsive to

subsequent oxytocin after intermittent compared to continuous exposure to oxytocin, most likely due to reduction in oxytocin receptor desensitization, or facilitation of receptor resensitization in the intermittent group[16]. The IPPH group were found to have longest duration of labor and highest neonatal weight, which could mean continuous and prolonged exposure to oxytocin might be used before treatment of IPPH thus resulting in desensitization, and lost expression of OTR in the IPPH group might be associated with this.

In IPPH with preconceived severe acidosis, hypoxia internal environment of myometrium cells, HIF-1 α , the key factor in the body's response to hypoxic stress, had similar significantly decreased expression as OTR compared to TNL and TAL group. In study of myocardial, HIF-1 α was found to inhibit the formation of AMP during hypoxia, reducing calcium overload and ROS produced by hypoxanthine metabolism to reduce myocardial damage[17]. And the transient hypoxia process was suggested to be an important mechanism of myometrium contraction in Alotaibi M research[18]. While myometrium need contractions at TAL group, increased HIF-1 α expression were shown compared to TNL group in this research, which might suggest self-protection as "hypoxia pre-conditioning" like in myocardial in order to regulate oxygen balance and contractile activity. But when meeting a prolonged labor in IPPH, decreased HIF-1 α expressions seemed lost related self-protection in uterine contraction force formation together with OTR lost expression.

But, ASIC1a, sensitive to pH level and Ca²⁺-H⁺ related channel, which is involved in the pathogenesis of central nervous system diseases[19], did not show obvious enhancement or weakening in the IPPH group as OTR. In study of musculoskeletal ASIC1a was considered a key and may be a pharmacological target for treatment of musculoskeletal pain when in acid environment[20]. This research results could remind us that acidosis state in myometrium previously expected might be not existing under IPPH or even MODS, DIC state. It might be related to the passive expansion of myometrium in third trimester and the local slightly acidic environment. And at the same time, the uterus which is at the low point of pelvis, always have rich blood circulation making acid-base balance easy to be in steady state.

At the same time, FATP and GLUT, also all did not show a significant change in the IPPH group. In research of myocardial contractile function, remodeling the metabolic pathways in chronic pathophysiological conditions, could result in modulations of myocardial energetics and contractile function, in which FATP and GLUT, represent glucose and lipid metabolism levels, always play an important role[21]. But even after a long period of labor and deadly IPPH state, this study showed us the myometrium could always get a stable metabolic ability and activity.

While apoptosis marker, Bax, Caspase-3, 9 showed a consistent change trend, which was significantly weakened at IPPH group, but Bcl-2 showed a consistent expression state. In many studies on ischemia and hypoxia injury of myocardial, upregulation of Bcl-2 is always considered as an important pathway target for inducing the protective function of myocardial cells[22] while Bax, Caspase play the opposite role[23]. The results in our research might mean that the myometrium could also always get a stable own repair ability and activity and without apoptosis or damage trend even in DIC, MODS state. Combined with

FATP and GLUR results, this research prompts us that the tolerance and plasticity of myometrium might be beyond our imagination. And it also showed that in the treatment of IPPH, effective surgical intervention to preserve uterus, a strong toughness and recovery of myometrium will guarantee the recovery and re-implantation ability of uterine, even experienced a fatal life blow in IPPH state.

Limitation

However, in this study, the initially founding was only based on semi-quantitative analysis of immunohistochemistry. Due to the very low incidence of IPPH, the difficulty of sampling in clinic, and the improvement of obstetric hemostasis techniques, the incidence of hysterectomy has become more and more rare in recent years. The research in the IPPH group can only rely on clinical pathological samples from the past 10 years, so the study itself may have certain limitations. Further research requires subsequent collection of living tissues and validation of PCR and WB research results.

Conclusion

Loss of OTR expression and HIF-1 self-protection were found in myometrium of intractable postpartum hemorrhage compared to normal term at or not at labour, which could reveal the etiology of the ineffectiveness of uterotonic agents and further pathogenesis and mechanism in IPPH (intractable postpartum hemorrhage) to a certain extent, thus needing timely surgical interventions or even hysterectomy to hemostasis for saving pregnancy lives.

Declarations

Ethics approval and consent to participate

This research was approved by the Ethics Committee of Guangzhou Women and Children Medical Center, and participants signed informed consent. Ethics number: No.2018041701.

Consent for publication

Not applicable

Acknowledgement

None

Competing interests

The authors declare that they have no competing interests" in this section.

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Authors' contributions

Yunshan Chen: Writing - Original Draft, Conceptualization, Methodology;

Lele Wang: Data Curation, Software, Validation, Formal analysis;

Minghua Hu: Data curation, Investigation, Resources, Visualization;

Feng Liu: Resources, Visualization;

Mingjing Deng: Data curation, Visualization;

Huishu Liu: Writing- Reviewing and Editing, Project administration, Funding acquisition;

All authors read and approved the final manuscript

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Declaration of Interest

The authors reported no conflicts of interest. The authors alone were responsible for the content and writing of the paper.

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Tables

Table 1

Immunoreactivity Scores

Score	Intensity	Positive rate
0	No staining	<5%
1	Faint staining	5-25%
2	Moderate staining	25-50%
3	Strong staining	50-75%
4		>75%

Table 2

Clinical Characteristics of the Study Participants

Parameters	TNL group	TAL group	IPPH group	P Value
Age (years)	34.9±2.6	35.4±2.5	35.3±2.0	P=0.181
Body mass index (BMI)	26.7 ± 0.9	26.5 ± 1.1	26.4±0.9	P=0.306
Gestational week (week)	39.1±0.8	39.4±1.1	39.8±0.8	P=0.872
Neonatal weight (grams)	3542 ± 227	3729 ±143	3977±111	P<0.01
Postpartum bleeding (ml)	307 ± 56	284 ± 79	3154±438	P<0.01
Duration of labor h	13.8±3.8	12.7±3.0	18.4±1.2	P<0.01

Table 3

OTR and acidosis, hypoxia factors expressions of myometrium in groups

group	score	0n,%	1n,%	2n,%	3+n,%	Z	P
OTR	TNL n,%	0(0%)	3(30%)	4(40%)	3(30%)	26.174	<0.01
	TAL n,%	0(0%)	2(20%)	4(40%)	4(40%)		
	IPPH n,%	9(90%)	1(10%)	0(0%)	0(0%)		
HIF-1α	TNL n,%	0(0%)	3(30%)	7(70%)	0(0%)	15.257	0.018
	TAL n,%	0(0%)	2(20%)	4(40%)	4(40%)		
	IPPH n,%	4(40%)	4(40%)	2(20%)	0(0%)		
ASIC1a	TNL n,%	0(0%)	3(30%)	5(50%)	2(20%)	1.000	0.910
	TAL n,%	0(0%)	3(30%)	6(60%)	1(10%)		
	IPPH n,%	0(0%)	4(40%)	4(40%)	2(20%)		

Table 4

Glucose and lipid metabolism expressions of myometrium in groups

group	score	0n,%	1n,%	2n,%	3+n,%	Z	P
FATP1	TNL n,%	1(10%)	4(40%)	5(50%)	0(0%)	0.725	0.948
	TAL n,%	2(20%)	3(30%)	5(50%)	0(0%)		
	IPPH n,%	2(20%)	4(40%)	4(40%)	0(0%)		
FATP4	TNL n,%	5(50%)	4(40%)	1(10%)	0(0%)	1.627	0.804
	TAL n,%	4(40%)	5(50%)	1(10%)	0(0%)		
	IPPH n,%	6(60%)	3(30%)	1(10%)	0(0%)		
GLUT2	TNL n,%	0(0%)	3(30%)	6(60%)	1(10%)	2.803	0.833
	TAL n,%	0(0%)	2(20%)	6(60%)	2(20%)		
	IPPH n,%	1(10%)	2(20%)	5(50%)	2(20%)		
GLUT4	TNL n,%	0(0%)	4(40%)	5(50%)	1(10%)	0.825	0.935
	TAL n,%	0(0%)	3(30%)	6(60%)	1(10%)		
	IPPH n,%	0(0%)	3(30%)	5(50%)	2(20%)		

Table 5

apoptosis factors expressions of myometrium in groups

group	score	- n,%	+ n,%	2 n,%	3+ n,%	Z	P
Bax	TNL n,%	0(0%)	1(10%)	5(50%)	4(40%)	20.250	0.002
	TAL n,%	1(10%)	3(30%)	6(60%)	0(0%)		
	IPPH n,%	5(50%)	4(40%)	1(10%)	0(0%)		
Bcl-2	TNL n,%	0(0%)	3(30%)	5(50%)	2(20%)	1.071	0.899
	TAL n,%	0(0%)	3(20%)	4(40%)	3(40%)		
	IPPH n,%	0(0%)	2(20%)	4(40%)	4(40%)		
Caspase3	TNL n,%	0(10%)	3(30%)	6(60%)	1(10%)	14.426	0.025
	TAL n,%	0(0%)	5(50%)	5(50%)	0(0%)		
	IPPH n,%	3(30%)	6(60%)	1(10%)	0(00%)		
Caspase9	TNL n,%	0(0%)	4(40%)	5(40%)	1(10%)	14.067	0.029
	TAL n,%	0(0%)	5(50%)	4(40%)	1(10%)		
	IPPH n,%	4(40%)	6(60%)	0(0%)	0(0%)		

Figures

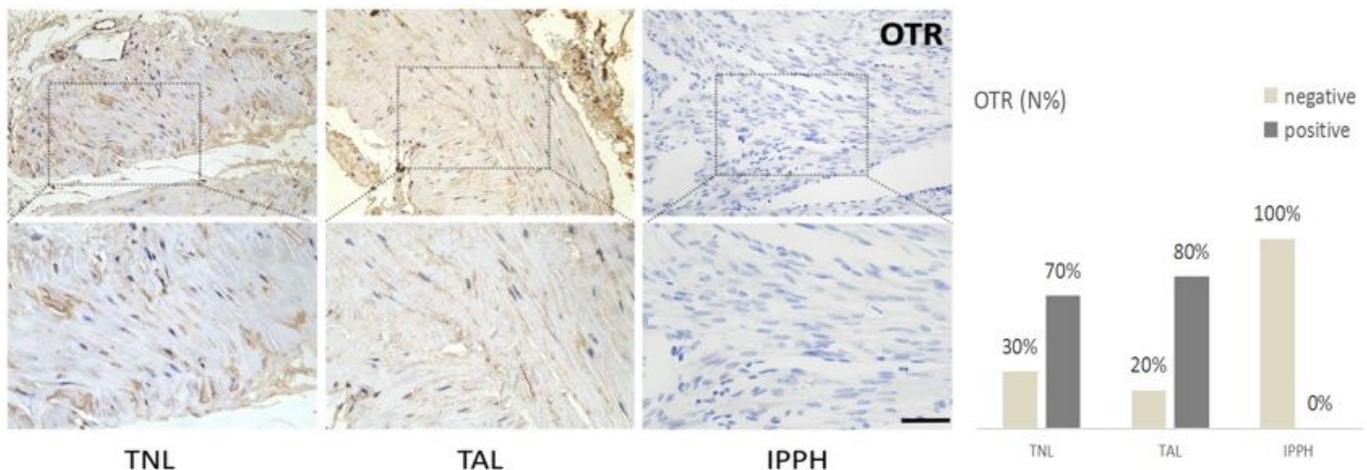


Figure 1

OTR expression in TNL group and TAL group, were moderately positive (40%,40%) and strongly positive (30%,40%). But in IPPH group, most expression was negative (90%) or weak positive (10%), with a

statistically difference ($p < 0.05$). TNL, term not in labour ;TAL, term in labour; IPPH, intractable postpartum hemorrhage; Scale bar= 25 μ m.

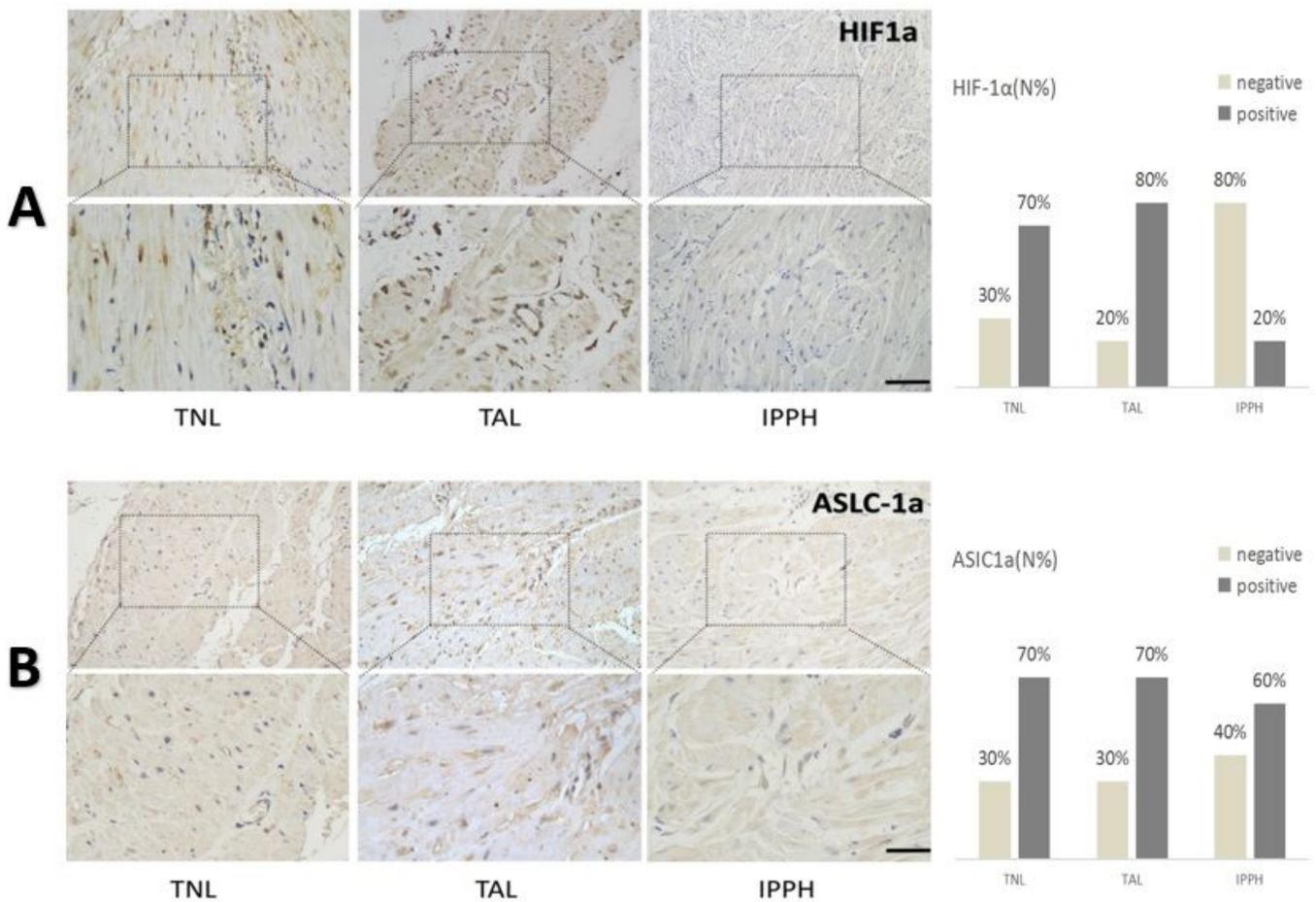


Figure 2

(A)The expression levels of HIF-1 α in TNL and TAL group were mostly moderately positive and strongly positive with rate of moderate and strong positive total were 70%, 80% compared to 20% in IPPH group, respectively statistically different ($p < 0.05$). (B) While, the positive expression of ASIC1a in myometrium was 70%(TNL), 80%(TAL), 60%(IPPH), with no significant difference ($P = 0.910$). TNL, term not in labour ;TAL, term in labour; IPPH, intractable postpartum hemorrhage; Scale bar= 25 μ m.

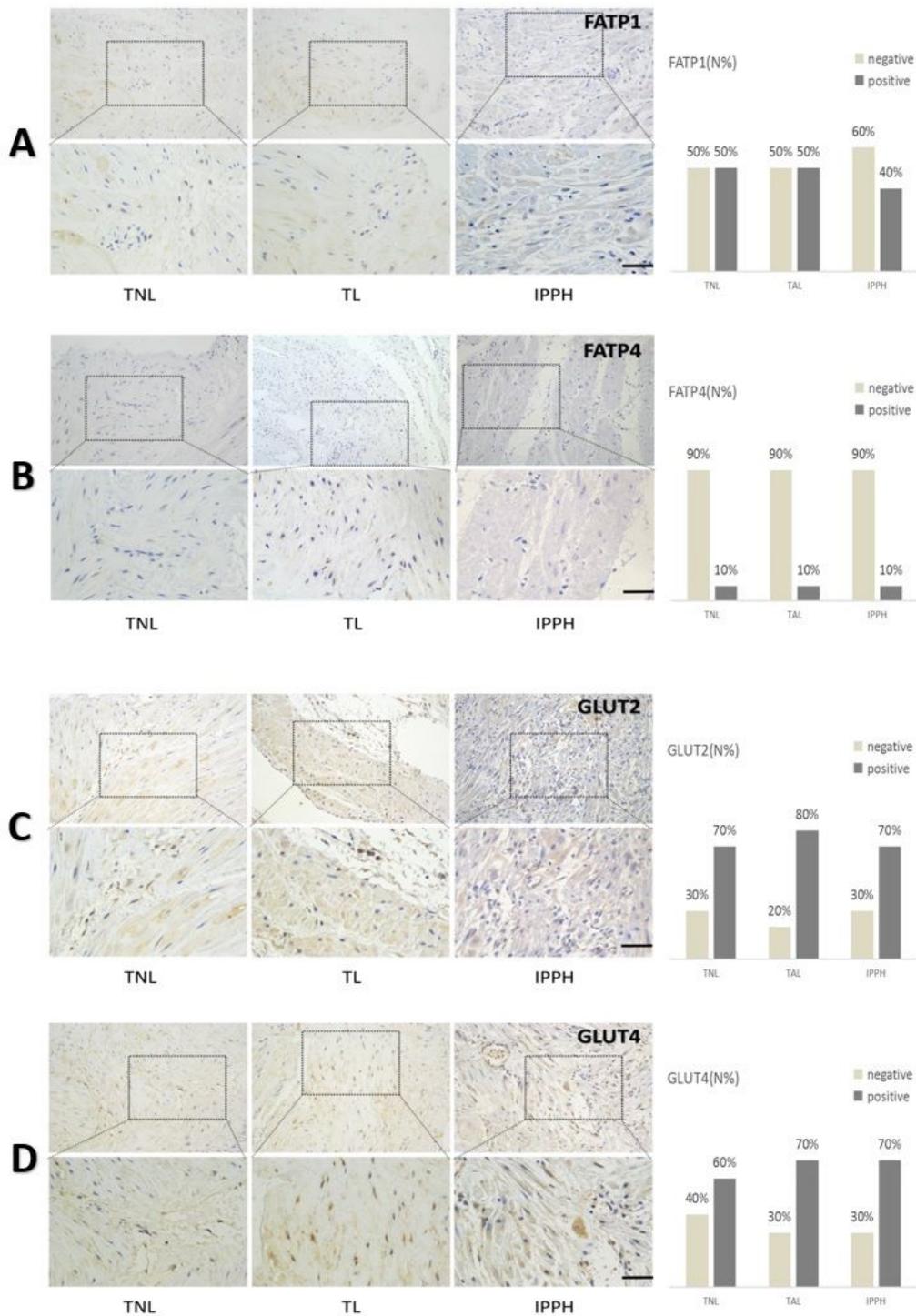


Figure 3

(A) FATP1 was expressed in 3 groups, with the positive rates of 50%(TNL) , 50%(TAL) , 40%(IPPH), respectively, with no statistical differences;(B)FATP4 had less positive expression on membrane, with the moderate positive levels of 10% in all three groups;(C,D)The positive expression rate of GLUT2 and GLUT4 were 70%,60%(TNL), 80%,70%(TAL), 70%, 70%(IPPH), respectively, all with no statistically

significant difference($P>0.05$).TNL, term not in labour ;TAL, term in labour; IPPH, intractable postpartum hemorrhage; Scale bar= 25 um.

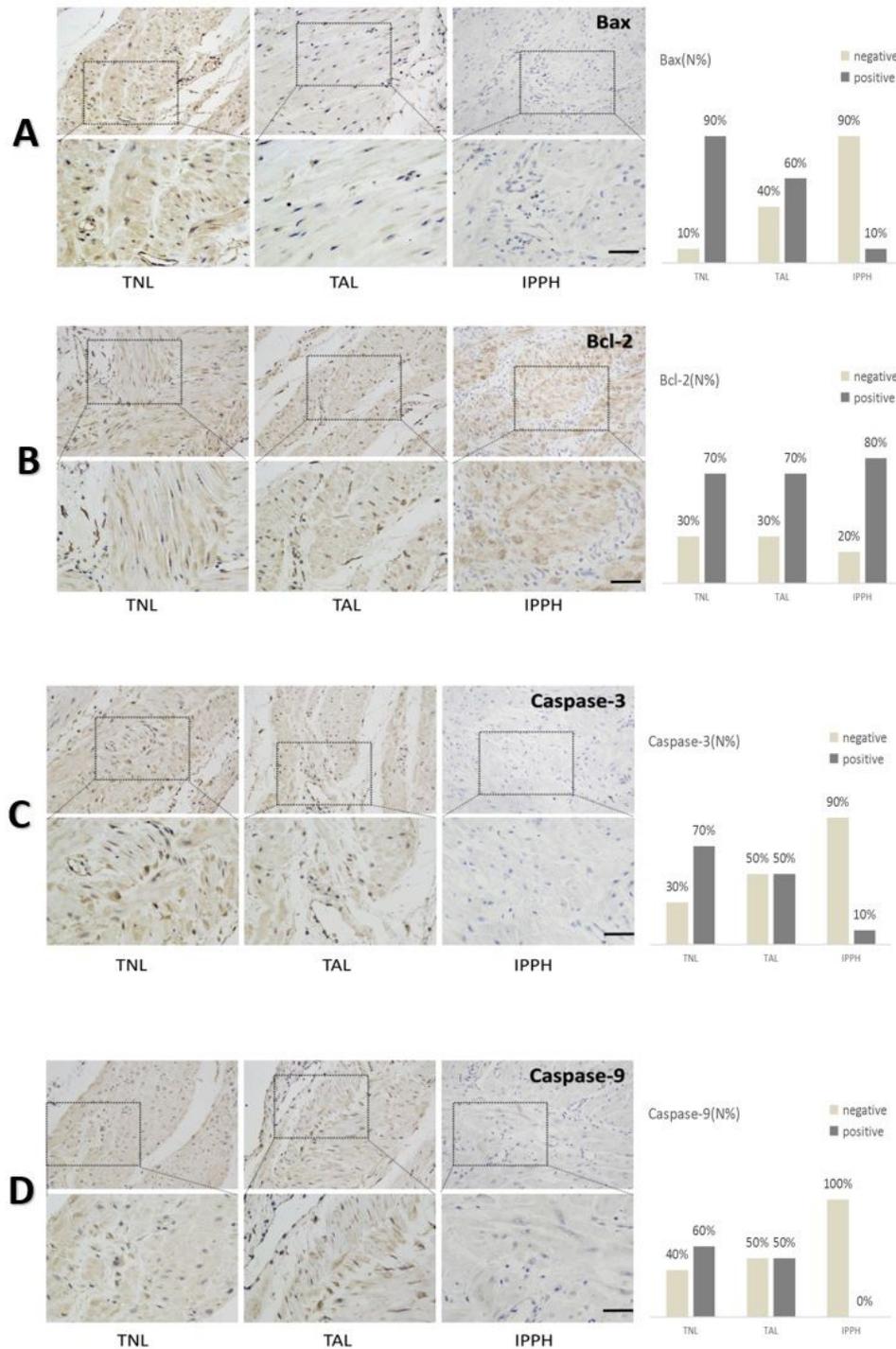


Figure 4

(A) Bax expression positive rate in IPPH group was 10%, significant decreased than TNL(90%) and TAL(90%) group; (B)the expression of Bcl-2 in the three groups all presented a high positive expression rate (TNL 70%, TAL 70%, IPPH 80%);(C,D) the expressions of caspase-3 and caspase-9 also showed the

same trend as Bax. The positive expression in IPPH group(10%,0%) were much weaker than TNL(70%,60%) and TAL(50%,50%) groups., respectively, with statistically significant difference($P < 0.05$). TNL, term not in labour ;TAL, term in labour; IPPH, intractable postpartum hemorrhage; Scale bar= 25 um.