Observational Study

Medicine®

OPEN

Biochemical clinical factors associated with missed abortion independent of maternal age

A retrospective study of 795 cases with missed abortion and 694 cases with normal pregnancy

Jie Fang, PhD[a], Bing Xie, MD[b], Binghai Chen, PhD[c], Chen Qiao, PhD[d], Bo Zheng, PhD[e], Xiaojin Luan, MD[a], Jiajia Liu, MD[a], Yidan Yan, MD[a], Qianwen Zheng, MD[a,g], Min Wang, MD[a], Wanyin Chen, MD[a], Zeyu He, MD[f], Cong Shen, MD[e], Hong Li, PhD[e,*], Xia Chen, MD[a,g,*], Jun Yu, PhD[a,g,*]

Abstract

The incidence of fertile women with missed abortion dramatically increased in recent years, while very few serum indices have been identified for the diagnosis of missed abortion. The aim of this study was to identify related factors for missed abortion through a retrospective study of serum indices.

A total of 795 cases of women with missed abortion and 694 cases of women with normal pregnancy between March 2014 and March 2017 were included in the present study. The diagnosis of missed abortion was based on clinical history, clinical examination, and transvaginal ultrasound findings. The final diagnosis of missed abortion was based on assessment of pregnancy structures (i.e., a gestational sac without fetal heart rate) via transvaginal ultrasound. We evaluated the clinical values of 4 serum indices and their relationship to missed abortion: gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), adenosine deaminase (ADA), and fibrinogen (FIB).

The serum levels of GGT, ADA, and FIB showed statistically significant differences comparing women who experienced missed abortion with women who had normal pregnancies (controls). Among women with missed abortion, the levels of GGT and ADA were dramatically increased (GGT: $P < .0001$; ADA: $P = .0459$), while FIB levels were slightly lower ($P = .0084$) compared to controls. The LDH levels exhibited a non-significant trend toward lower levels in the missed abortion group ($P = .3951$). Interestingly, the observed significant increase in serum GTT levels among women with missed abortion was not affected by maternal age.

This study found that GTT may be a useful marker which was associated with missed abortion, indicating its potential clinical roles in missed abortion.

Editor: Daryle Wane.

Jie Fang, Bing Xie, Binghai Chen, Chen Qiao and Bo Zheng: these authors contributed equally to this work.

Ethics approval and consent to participate: All participants in this study were fully informed of the purpose and significance of the study. The project was approved by Medical Ethics Committee at the Fourth People's Hospital of Zhenjiang and Ethics Committee for Biomedical Research at Affiliated Hospital of Jiangsu University, and conducted according to the guide for clinical retrospective studies.

Funding: This work was supported by National Natural Science Foundation of China (grant numbers 31701298, 81402100), Natural Science Foundation of Jiangsu Province (BK20170562, BK20170564), Key Research Foundation of Zhenjiang Social Development (grant numbers SH2018065, SH2017013, SH2017020, SH2016028, SH2016031, SH2014026), Key Research Foundation of Zhenjiang Health Science and Technology (grant number SHW2016001), Science Foundation of Doctorate Research of Affiliated Hospital of Jiangsu University (grant number jdfyRC2016005, jdfyRC2016003), the Foundation of Health and Family Planning Commission of Jiangsu Province (grant number Q201408), the Foundation for Young Medical Talents of Jiangsu province (grant number QNRC2016840), Six Talent Peaks Project in Jiangsu Province (grant number WSW-007), Suzhou Key Medical Center (grant number SZZX201505), Suzhou Introduced Project of Clinical Medical Expert Team (grant number SZYJTD201708) and Jiangsu Provincial Medical Innovation Team (grant number CXTDB2017013).

Supplemental Digital Content is available for this article.

The authors have no conflicts of interest to disclose.

[a] Department of Gynecology, the Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, [b] Department of Obstetrics and Gynecology, the Fourth People's Hospital of Zhenjiang, Zhenjiang, [c] Department of Urology, Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, [d] Department of Clinical Pharmacy, Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, [e] Center for Reproduction and Genetics, Suzhou Municipal Hospital, the Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, [f] Department of Clinical Medicine, China Medical University, Shenyang, [g] Reproductive Sciences Institute of Jiangsu University, Jiangsu University, Zhenjiang, China.

[*] Correspondences: Jun Yu, Department of Gynecology, the Affiliated Hospital of Jiangsu University; Reproductive Sciences Institute of Jiangsu University, Jiangsu University, Zhenjiang Jiangsu 212001, China (e-mail: yujun9117@126.com), Xia Chen, Department of Gynecology, the Affiliated Hospital of Jiangsu University; Reproductive Sciences Institute of Jiangsu University, Jiangsu University, Zhenjiang Jiangsu 212001, China (e-mail: chenxia_0511@126.com), Hong Li, Center for Reproduction and Genetics, Suzhou Municipal Hospital, the Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Jiangsu 215002, China (e-mail: hongliszivf@163.com).

Medicine (2018) 97:50(e13573)

Received: 30 August 2018 / Accepted: 15 November 2018

http://dx.doi.org/10.1097/MD.0000000000013573

Abbreviation: ADA = adenosine deaminase, CI = confidence interval, DCs = decidual cells, Dll4/Notch = Delta-like ligand 4/Notch, EPL = early pregnancy loss, ER = endoplasmic reticulum, FAIM = Fas inhibitory molecule, FIB = fibrinogen, GGT = gamma-glutamyltransferase, HIF-1a = Hypoxia inducible factor 1a, IDO = indoleamine 2,3-dioxygenase, LDH = lactate dehydrogenase, MA = missed abortion, miR-575 = microRNA 575, ORs = odds ratios, S100A11 = S100 calcium-binding protein A11, SD = standard deviation, TGFβ1 = transforming growth factor β1, TIPE2 = Tumor necrosis factor-α-induced protein-8 like-2, TVU = transvaginal ultrasound, UBE2N = ubiquitin-conjugating enzyme E2N, VEGF = Vascular endothelial growth factor.

Keywords: adenosine deaminase, biomarker, fibrinogen, gamma-glutamyltransferase, lactate dehydrogenase, missed abortion

1. Introduction

Miscarriage, also known as spontaneous abortion, is a common complication in pregnancy and there is still a lack of biomarkers with predictive value for asymptomatic patients before the event occurs. Missed abortion (MA) is a specific type of miscarriage, and refers to embryonic or fetal death with failure of the retained intrauterine products of conception to be discharged naturally. Women with MA may have no obvious symptoms, yet it occurs in approximately 8 to 20% of clinically diagnosed pregnancies.[1–2] The MA may cause maternal morbidity, including endometrial injury, coagulative dysfunction, depression, and anxiety. Presently, multiple etiologic factors including parental chromosomal abnormalities, immunological factors, endocrine disorders, uterine abnormalities, hereditary thrombophilia, infections, and environmental factors have been identified for MA, and these conditions may occur in up to 50% of all women with miscarriages.[3]

Recently, a few gene expression and functional studies revealed a correlation between genetic factors and MA. Evidences indicated that early gestation is associated with a hypoxic environment, which may encourage angiogenesis, but severe hypoxia may inhibit angiogenesis. Placental angiogenesis is dependent upon various growth factors (including VEGF and its receptors, and so on). For instance, aberrant Delta-like ligand 4/Notch (Dll4/Notch) and Hypoxia inducible factor 1a/Vascular endothelial growth factor (HIF-1a/VEGF) signaling may have a role in MA.[4] The expression of HIF-1α and VEGF was lower in the MA, and the levels of HIF-1α/VEGF mRNA and protein in HTR8/SVneo cells were significantly enhanced under hypoxia.[5] Moreover, microRNA 575 (miR-575) has been demonstrated to be upregulated in maternal placenta in patients who have experienced a miscarriage. Abnormal expression of miR-575 may lead to MA by influencing apoptosis and angiogenesis. Inhibition of miR-575 may inhibit apoptosis and promote angiogenesis in MA.[6] Tumor necrosis factor-α-induced protein-8 like-2 (TIPE2), identified as a member of the TNFAIP8 family, is a negative regulator of inflammation and immunity.[7,8] A TIPE2 could play important roles in maintaining maternal-fetal tolerance, and decreased TIPE2 expression in the decidua may be related to the development of MA.[9] Another study aimed to explore the expression of suppressor of cytokine signaling (SOCS3), transforming growth factor β1 (TGFβ1), and indoleamine 2,3-dioxygenase (IDO), and to analyze the association between SOCS3 and TGFβ with IDO expression in chorionic villi and decidua at the maternal-fetal interface during early pregnancy. In the normal physiological state of pregnancy, SOCS3 and TGFβ may be involved in the regulation of immune tolerance by positive or negative regulation of IDO expression at the maternal-fetal interface.[10] Taken together, these studies indicated that many biological processes (i.e. angiogenesis, hypoxia, apoptosis, inflammation, and immunity) and their regulatory pathways are crucial for the pathogenicity of MA. And these studies begin to offer some insights, but the exact mechanism of early pregnancy loss in MA remains to be elucidated. However, none of them have analyzed the role of plasma metabolites as promising biomarkers of MA.

Using UHPLC-MS based metabolomics screening, plasma metabolic profiles identify plasma metabolite biomarkers (i.e. glyceric acid, indole, and sphingosine) as having perfect accuracy for diagnosis of MA, which make it possible to explore promising biomarkers in plasma.[11] Here, in this study, 4 serum indices represent markers for metabolic homeostasis of the human body and are involved in oxidative stress reaction, apoptosis, and multiple metabolic processes. Gamma-glutamyltransferase (GGT) is a transferase that catalyzes the transfer of gamma-glutamyl functional groups from molecules such as glutathione to acceptor molecules, forming glutamate.[12] The GGT induction has been shown to be related to oxidative stress and apoptosis[13] also GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification.[14] Adenosine deaminase (ADA) is an enzyme involved in purine metabolism. It plays a key function in the development and maintenance of the immune system in humans.[15] It has also been recognized that the activity of ADA protein is upregulated in mouse hearts overexpressing HIF1a, suggesting a role in oxygen-related homeostatic signaling.[16] Lactate dehydrogenase (LDH) is an enzyme found in nearly all living cells, and is broadly expressed in body tissues including blood cells and heart muscle. The LDH is a valuable biomarker for common injuries and disease based on its release during tissue damage. Fibrinogen (FIB) is a glycoprotein that in vertebrates circulates in the blood. A study with a small population showed that serum FIB levels showed a small but non-significant decrease in women with MA.[17] According to long-term clinical observations, these serum indices may be influenced by the pathogenesis of MA.

The aim of this study was to identify potential predictive factors for MA through a retrospective study of serum indices. In this study, we systemically analyzed the association between 4 serum indices (GGT, ADA, LDH, and FIB) and MA in order to assess whether they could be used as early prediction factor(s) for MA.

2. Materials and methods

2.1. Study design and participants

This study was a retrospective study. A total of 795 cases of women aged from 17 to 49 years with MA at 7 to 10 gestational weeks at The Fourth People's Hospital of Zhenjiang (Zhenjiang, China) between March 2014 and March 2017 were included in the present study. An additional 694 cases of fertile women aged between 17 and 48 years with no history of MA were used as controls.

The diagnosis of MA was based on the clinical history, clinical examination, and transvaginal ultrasound (TVU) results. In cases

Table 1

Characteristics of study population: women with normal pregnancy and women with MA.

	Control	MA	P
Participants, n	694	795	
Age, y, Mean±SD	29.99±6.36	30.55±5.74	.0772
17–27 (y), n (%)	282 (40.64%)	275 (34.59%)	
28–38 (y), n (%)	333 (47.98%)	431 (54.21%)	
39–49 (y), n (%)	79 (11.38%)	89 (11.20%)	
Lower abdominal pain, n (%)	29 (4.18%)	34 (4.28%)	.9253
Vaginal bleeding, n (%)	5 (0.72%)	6 (0.75%)	.9386

The data were evaluated for statistical differences using student's *t* test. MA = missed abortion, SD = standard deviation.

where pregnancy structures (a gestational sac without fetal heart rate) were identified by TVU, the final diagnosis of MA was made. In control group, pregnancy structures are normal.

Inclusion criteria were a gestational age at 7 to 10 weeks (based on the 1st day of the last menstrual period) and no history of recurrent spontaneous abortions, chromosomal abnormalities, endocrine diseases, anatomical abnormalities of genital tract, infections, immunologic diseases, trauma, internal diseases, hereditary disorders, maternal diseases, psychological factors, or any chemical agent intake before their elective terminations.[18,19] No obvious other internal medical and surgical disease was found in both groups.

2.2. Definition of age groups

For reducing possible biases affecting the relationship between serum levels and MA, the overall study population was divided into different age groups, including age group 1 (17–27 years), age group 2 (28–38 years) and age group 3 (39–49 years).

2.3. Detection of serum indices

The samples (5 ml) poured into the testing tube without anticoagulant, to divide serum. In order to decrease the time of keeping samples in the laboratory conditions, during 5 min of incubation in the environmental temperature, the sample immediately became centrifuged (4000 rpm for 5 min) and the serum solution was divided by blood clot. Then the resulted serum was used to test serum levels of GGT, LDH, ADA, and FIB. Serum indices were measured at the same time point with an automated biochemical analyzer (Beckman Coulter AU5800).

2.4. Statistical analysis

Values were measured using the mean with standard deviation (SD). Differences between 2 groups were calculated by student's *t* test. Odds ratios (ORs) and 95% confidence interval (CI) were calculated by using logistic regression. Data were analyzed by using SPSS software, version 22 (IBM, IL) and PASS software, Version 11.0.7 (NCSS).

3. Results

3.1. Study populations

Our retrospective study includes 694 cases of healthy fertile women with normal pregnancies and 795 cases of women with MA. Mean age between control (30.0±6.4 years) and MA (30.6 ±5.7 years) subjects showed no statistically significant difference ($P=.08$). Twenty-nine cases (4.2%) in the control group and 34 cases (4.3%) in the MA group had lower abdominal pain ($P=.93$). Five cases (0.7%) in the control group and 6 cases (0.8%) in the MA group had at least 1 occurrence of vaginal bleeding ($P=.94$). A comparison of baseline general and gynecologic characteristics among the control and MA groups is summarized in Table 1.

3.2. Serum indices analysis identifies 3 potential biomarkers for missed abortion

Our results show that the levels of serum GGT and ADA significantly increased in women with MA (GGT: 17.68±9.85 IU/L; ADA: 6.05±3.94 IU/L) when compared to women with normal pregnancies (GGT: 14.57±7.93 IU/L, $P<.0001$; ADA: 5.67±3.36 IU/L, $P=.0459$). Moreover, serum FIB levels were modestly but significantly lower in the MA group compared to controls (MA: 4.31±0.91 g/L; control: 4.44±0.96 g/L; $P=.0084$). Serum LDH levels did not differ among the MA and control groups (MA: 148.05±30.46 IU/L; control: 149.34± 27.88 IU/L, $P=.3951$). Taken together, these data suggest that 3 out of the 4 key metabolism-related biochemical indicators evaluated in this study (GGT, ADA, and FIB) may be potential predictive markers for MA (Table 2).

Table 2

Comparison of 4 serum indices in women with normal pregnancy and women with MA.

	Control		MA			
	n	Mean±SD	n	Mean±SD	OR (95% CI)	P
GGT (IU/L)	693	14.57±7.93	793	17.68±9.85	1.046 (1.031–1.061)	<.0001
LDH (IU/L)	694	149.34±27.88	795	148.05±30.46	0.998 (0.995–1.002)	.3951
ADA (IU/L)	693	5.67±3.36	793	6.05±3.94	1.040 (0.998–1.084)	.0459
FIB (g/L)	694	4.44±0.96	795	4.31±0.91	0.863 (0.773–0.964)	.0084

The data were evaluated for statistical differences using student's *t* test, ORs and 95% CI were calculated by using logistic regression. ADA = adenosine deaminase, CI = confidence interval, FIB = fibrinogen, GGT = gamma-glutamyltransferase, LDH = lactate dehydrogenase, MA = missed abortion, ORs = odds ratios, SD = standard deviation.

Table 3

Comparison of 4 serum indices by age in women with normal pregnancy (control) and women with missed abortion (MA).

	Control		MA			
Age group 1 (17–27 y)	**n**	**Mean±SD**	**n**	**Mean±SD**	**OR (95% CI)**	***P***
GGT (IU/L)	282	13.97±7.46	274	16.47±7.89	1.049 (1.022–1.076)	.0001
LDH (IU/L)	282	148.46±28.90	275	146.67±25.40	0.998 (0.991–1.004)	.4382
ADA (IU/L)	281	5.67±2.12	275	6.15±3.22	1.082 (1.002–1.168)	.0378
FIB (g/L)	282	4.50±0.92	275	4.26±0.79	0.720 (0.589–0.880)	.0011
	Control		**MA**			
Age group 2 (28–38 y)	**n**	**Mean±SD**	**n**	**Mean±SD**	**OR (95% CI)**	***P***
GGT (IU/L)	333	14.58±7.65	430	17.92±10.43	1.049 (1.028–1.070)	<.0001
LDH (IU/L)	333	147.84±25.59	431	148.10±32.50	1.000 (0.995–1.005)	.904
ADA (IU/L)	333	5.65±4.29	429	5.98±4.58	1.021 (0.979–1.065)	.3095
FIB (g/L)	333	4.39±1.03	431	4.36±1.00	0.963 (0.837–1.109)	.6051
	Control		**MA**			
Age group 3 (39–49 y)	**n**	**Mean±SD**	**n**	**Mean±SD**	**OR (95% CI)**	***P***
GGT (IU/L)	78	16.71±10.18	89	20.27±11.76	1.033 (1.000–1.066)	.0391
LDH (IU/L)	79	158.80±31.71	89	152.02±34.36	0.994 (0.984–1.003)	.1879
ADA (IU/L)	79	5.75±2.30	89	6.09±2.38	1.065 (0.933–1.216)	.349
FIB (g/L)	79	4.41±0.74	89	4.25±0.83	0.768 (0.520–1.136)	.186

The data were evaluated for statistical differences using student's *t* test, ORs and 95% CI were calculated by using logistic regression. ADA = adenosine deaminase, CI = confidence interval, FIB = fibrinogen, GGT = gamma-glutamyltransferase, LDH = lactate dehydrogenase, MA = missed abortion, ORs = odds ratios, SD = standard deviation.

3.3. GGT levels in MA are independent of maternal age

Genetic and metabolic risk factors increase significantly with age. To explore early predictive factors that are independent of age, we divided our study population into 3 age groups, comprising 17 to 27 years, 28 to 38 years, and 39 to 49 years.

Among age-specified subgroups, 3 serum indices exhibited extremely similar serum level patterns among women ages from 17 to 49 years. The serum concentrations of GGT and ADA significantly increased, while FIB levels were decreased among women with MA. Surprisingly, a similar pattern was observed for women ages from 17 to 27 years. On the other hand, mean GTT serum level was significantly increased among all age groups of women with MA (Table 3). Our results demonstrate that GTT may be a potential predictive factor for MA independent of maternal age.

4. Discussion

The MA is one of the most common types of early pregnancy loss (EPL), and several reasons have been identified for the failure of these pregnancies. The incidence of fertile women with MA dramatically increased in the last few years, while very few serum indices have been identified for the diagnosis of MA. In this study, we initially started with 4 candidate serum indices (GGT, ADA, FIB, and LDH) and evaluated the relationship between clinical values of these serum indices and MA (Table S1, http://links.lww.com/MD/C697 and Table S2, http://links.lww.com/MD/C697). Comparing women who experienced MA with control women who had healthy pregnancies, the serum enzyme levels of GGT, ADA, and FIB showed statistically significant differences. Surprisingly, although GGT levels remained within the normal range (8–87 IU/L), these levels were significantly higher in women with MA compared to controls, independent of age. These observations demonstrate for the 1st time that GGT serum level may be a novel predictive biomarker for the diagnosis of MA.

The GGT enzyme is widely distributed in the human body, including the kidneys, bile duct, pancreas, gallbladder, spleen, heart, brain, and seminal vesicles, and is frequently localized to the plasma membrane with its active site directed toward the extracellular space.[12,20] Serum GGT activity has already been used as a biomarker for liver diseases or alcohol consumption in clinical practice. However, serum GGT is more than a marker of liver diseases or alcohol consumption. Epidemiological studies have found a strong association between serum GGT levels and many cardiovascular disease risk factors.[21,22] In addition, several prospective studies have shown that baseline serum GGT level is an independent risk factor for the development of heart disease, hypertension, stroke, and type 2 diabetes.[21–25]

An increase of serum GGT might be interpreted as a defense mechanism reflecting the induction of cellular GGT activity under oxidative stress.[26,27] There is evidence that cellular GGT plays an important role in antioxidant defense systems and mediates oxidative stress- induced cell death.[28,29] Other studies show that the increased resistance of GGT-expressing cells to H_2O_2-induced apoptosis results from activation of ASK-1/p38 signaling and increased expression of cellular catalase, resulting in decreased formation of reactive oxygen species (ROS) and thereby protection against ROS-induced DNA damage.[30,31]

The correlation between mRNA and protein levels is typically insufficient to predict protein expression levels.[32] A previous study using 2D gel-based proteomics identified only 13 proteins dysregulated in placental villous tissues of EPL: Fas inhibitory molecule (FAIM), S100 calcium-binding protein A11 (S100A11), and RNA-binding protein regulatory subunit were down-regulated, and 5 proteins, including ubiquitin-conjugating enzyme E2N (UBE2N) and the proteasome beta-subunit were significantly upregulated.[18] Ni et al conducted a proteomics analysis to identify differentially expressed proteins in placental villous tissues from normal pregnant women and EPL patients, and found 51 differentially expressed proteins, of which 22 proteins were upregulated and 29 proteins were downregulated; these proteins mainly participate in cell migration, angiogenesis, oxidative stress, apoptosis, and metabolic pathways.[19] Another study performed by Xin et al identified 5952 proteins in placental villi, 588 of which were differentially expressed in women who

experienced EPL. Further bioinformatics analysis indicated that these differentially expressed proteins participated in a variety of signaling pathways, including the focal adhesion pathway and ribosome pathway.[33] These identified proteins, especially those with known roles in apoptosis and oxidative stress, may be novel predictive markers for MA.

Several studies suggest that regulation of apoptosis is critically important for the successful development and outcome of a pregnancy.[34–36] Low rates of apoptosis in placental villi tissues are a normal physiologic phenomenon,[37] while high levels of apoptosis may result in miscarriage.[38,39] Oxidative stress is a common pathological background for different etiologies of EPL, and it has been suggested that elevated ROS trigger endoplasmic reticulum (ER) stress by influencing ER function.[18,40] Accumulation of ROS and regulation of protein folding are closely linked events, and alterations in redox status or generation of ROS can affect ER homeostasis and protein folding.[41] The ER stress induces apoptosis in part via activation of caspase-4 and caspase-12.[42,43] Liu et al provided evidence that sustained ER stress occurs in EPL decidual cells (DCs), and the potentially deleterious relationship between ER stress and oxidative stress is likely to play an important role in the development of EPL.[43]

While providing important insights into the etiology of EPL, none of the aforementioned studies systematically evaluated serum analytes in women with EPL. Additionally, our study assessed GTT levels during an earlier stage of gestation. Our study demonstrates that apoptosis and oxidative stress-related serum index GTT may improve the efficiency of diagnosis of MA, representing a new and possibly independent tool in prediction assessment. Additional investigation of this relationship with larger populations will help to refine the present findings. Gynecologists and obstetricians should be aware of the potential added value of GTT for counseling and predicting risk of MA during pregnancy. Our study demonstrates that GTT may be a useful marker associated with MA and may improve the diagnostic efficiency of MA.

Acknowledgments

The authors wish to thank all patients for their participation and thank all research staff, and students who assisted with this work.

Author contributions

Conceptualization: Jie Fang, Jun Yu.
Data curation: Binghai Chen.
Formal analysis: Chen Qiao, Bo Zheng, Zeyu He.
Investigation: Xiaojin Luan.
Methodology: Jiajia Liu, Yidan Yan.
Project administration: Jie Fang, Bing Xie, Jun Yu.
Resources: Bing Xie, Qianwen Zheng, Cong Shen.
Software: Wanyin Chen, Zeyu He.
Supervision: Xiaojin Luan.
Validation: Min Wang, Cong Shen, Xia Chen.
Writing – original draft: Jie Fang, Xia Chen, Jun Yu.
Writing – review & editing: Hong Li, Jun Yu.

References

[1] Wood SL, Brain PH. Medical management of missed abortion: a randomized clinical trial. Obstet Gynecol 2002;99:563–6.
[2] Chen BA, Creinin MD. Contemporary management of early pregnancy failure. Clin Obstet Gynecol 2007;50:67–88.
[3] Fang Y, Kong B, Yang Q, et al. MDM2 309 polymorphism is associated with missed abortion. Hum Reprod 2009;24:1346–9.
[4] Fang Y, Yu S, Ma Y, et al. Association of Dll4/notch and HIF-1a -VEGF signaling in the angiogenesis of missed abortion. PLoS One 2013;8: e70667.
[5] Zhi Z, Yang W, Liu L, et al. Early missed abortion is associated with villous angiogenesis via the HIF-1α/VEGF signaling pathway. Arch Gynecol Obstet 2018;298:537–43.
[6] Xia S, Zhen Y, Ma H, et al. Abnormal expression of microRNA-575 leads to missed abortion through regulating apoptosis and angiogenesis. Exp Ther Med 2017;14:3993–4000.
[7] Sun H, Gong S, Carmody RJ, et al. TIPE2, a negative regulator of innate and adaptive immunity that maintains immune homeostasis. Cell 2008;133:415–26.
[8] Wang Z, Fayngerts S, Wang P, et al. TIPE2 protein serves as a negative regulator of phagocytosis and oxidative burst during infection. Proc Natl Acad Sci U S A 2012;109:15413–8.
[9] Sun Y, Wang X, Li Y, et al. The decreased expression of TIPE2 protein in the decidua of patients with missed abortion and possible significance. Reprod Biol Endocrinol 2017;15:68.
[10] Liu W, Huang Y, Huang G, et al. Relationship of SOCS3 and TGF-β with IDO expression in early pregnancy chorionic villi and decidua. Exp Ther Med 2017;14:4817–24.
[11] Fei H, Hou J, Wu Z, et al. Plasma metabolomic profile and potential biomarkers for missed abortion. Biomed Chromatogr 2016;30:1942–52.
[12] Whitfield JB. Gamma glutamyl transferase. Crit Rev Clin Lab Sci 2001;38:263–355.
[13] Pompella A, Paolicchi A, Corti A, et al. Gamma-glutamyltransferase, H2O2-induced apoptosis and expression of catalase. Toxicol In Vitro 2013;27:991.
[14] Courtay C, Oster T, Michelet F, et al. Gamma-glutamyltransferase: nucleotide sequence of the human pancreatic cDNA. Evidence for a ubiquitous gamma-glutamyltransferase polypeptide in human tissues. Biochem Pharmacol 1992;43:2527–33.
[15] Wilson DK, Rudolph FB, Quiocho FA. Atomic structure of adenosine deaminase complexed with a transition-state analog: understanding catalysis and immunodeficiency mutations. Science 1991;252:1278–84.
[16] Wu J, Bond C, Chen P, et al. HIF-1α in the heart: remodeling nucleotide metabolism. J Mol Cell Cardiol 2015;82:194–200.
[17] Mollamahmutoğlu L, Kalyoncu S, Engin-Ustün Y, et al. Troponin I, C-reactive protein and fibrinogen levels in missed abortions. Clin Exp Obstet Gynecol 2011;38:60–2.
[18] Liu AX, Jin F, Zhang WW, et al. Proteomic analysis on the alteration of protein expression in the placental villous tissue of early pregnancy loss. Biol Reprod 2006;75:414–20.
[19] Ni X, Li X, Guo Y, et al. Quantitative proteomics analysis of altered protein expression in the placental villous tissue of early pregnancy loss using isobaric tandem mass tags. Biomed Res Int 2014;2014:647143.
[20] Lee DH, Blomhoff R, Jacobs DRJr. Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res 2004;38:535–9.
[21] Lee DH, Ha MH, Kim JH, et al. Gammaglutamyltransferase and diabetes–a 4 year follow-up study. Diabetologia 2003;46:359–64.
[22] Lee DH, Jacobs DRJr, Gross M, et al. Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Clin Chem 2003;49:1358–66.
[23] Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. Diabetes Care 1998;21:732–7.
[24] Miura K, Nakagawa H, Nakamura H, et al. Serum gamma-glutamyl transferase level in predicting hypertension among male drinkers. J Hum Hypertens 1994;8:445–9.
[25] Jousilahti P, Rastenyte D, Tuomilehto J. Serum gamma-glutamyl transferase, self-reported alcohol drinking, and the risk of stroke. Stroke 2000;31:1851–5.
[26] Lee DH, Gross MD, Jacobs DRJr. Cardiovascular Risk Development in Young Adults StudyAssociation of serum carotenoids and tocopherols with gamma-glutamyltransferase: the Cardiovascular Risk Development in Young Adults (CARDIA) study. Clin Chem 2004;50:582–8.
[27] Lee DH, Steffen LM, Jacobs DRJr. Association between serum gamma-glutamyltransferase and dietary factors: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Am J Clin Nutr 2004;79:600–5.
[28] Kugelman A, Choy HA, Liu R, et al. Gamma-glutamyl transpeptidase is increased by oxidative stress in rat alveolar L2 epithelial cells. Am J Respir Cell Mol Biol 1994;11:586–92.

[29] Karp DR, Shimooku K, Lipsky PE. Expression of gamma-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. J Biol Chem 2001;276:3798–804.
[30] Giommarelli C, Corti A, Supino R, et al. Cellular response to oxidative stress and ascorbic acid in melanoma cells overexpressing gamma-glutamyltransferase. Eur J Cancer 2008;44:750–9.
[31] Giommarelli C, Corti A, Supino R, et al. Gamma-glutamyltransferase-dependent resistance to arsenic trioxide in melanoma cells and cellular sensitization by ascorbic acid. Free Radic Biol Med 2009;46:1516–26.
[32] Gygi SP, Rochon Y, Franza BR, et al. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 1999;19:1720–30.
[33] Xin L, Xu B, Ma L, et al. Proteomics study reveals that the dysregulation of focal adhesion and ribosome contribute to early pregnancy loss. Proteomics Clin Appl 2016;10:554–63.
[34] Savion S, Lepsky E, Orenstein H, et al. Apoptosis in the uterus of mice with pregnancy loss. Am J Reprod Immunol 2002;47:118–27.
[35] Choi HK, Choi BC, Lee SH, et al. Expression of angiogenesis-and apoptosis-related genes in chorionic villi derived from recurrent pregnancy loss patients. Mol Reprod Dev 2003;66:24–31.
[36] Chen H, Deng X, Yang Y, et al. Expression of GRIM-19 in missed abortion and possible pathogenesis. Fertil Steril 2015;103:138–46.
[37] Halperin R, Peller S, Rotschild M, et al. Placental apoptosis in normal and abnormal pregnancies. Gynecol Obstet Invest 2000;50:84–7.
[38] Cinar O, Kara F, Can A. Potential role of decidual apoptosis in the pathogenesis of miscarriages. Gynecol Endocrinol 2012;28:382–5.
[39] Nair RR, Khanna A, Singh K. Association of FAS-1377 G>A and FAS-670 A>G functional polymorphisms of FAS gene of cell death pathway with recurrent early pregnancy loss risk. J Reprod Immunol 2012;93:114–8.
[40] Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. Hum Reprod Update 2006;12:747–55.
[41] Malhotra JD, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid Redox Signal 2007;9:2277–93.
[42] Nishitsuji K, Tomiyama T, Ishibashi K, et al. The E693Delta mutation in amyloid precursor protein increases intracellular accumulation of amyloid beta oligomers and causes endoplasmic reticulum stress-induced apoptosis in cultured cells. Am J Pathol 2009;174:957–69.
[43] Liu AX, He WH, Yin LJ, et al. Sustained endoplasmic reticulum stress as a cofactor of oxidative stress in decidual cells from patients with early pregnancy loss. J Clin Endocrinol Metab 2011;96:E493–7.