JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2022, 73, 2, 261-268
www.jpp.krakow.pl | DOI: 10.26402/jpp.2022.2.09

Z. LI[1,2], L. LEI[3], L. LING[1], Y. LIU[1], Z. XIONG[1], Y. SHAO[3]

RESVERATROL MODULATES THE GUT MICROBIOTA OF CHOLESTASIS IN PREGNANT RATS

[1]Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Chongqing Medical University, Yuzhong District, Chongqing, China; [2]The Department of Obstetrics and Gynecology, BiShan Hospital of Chongqing Medical University, Bishan District, Chongqing, China; [3]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Chongqing Medical University, Yuzhong District, Chongqing, China

This study aims to investigate the effect of resveratrol on intrahepatic cholestasis of pregnancy (ICP) and its effect on the gut microbiome profiles, thus contributing to the potential therapeutic strategies for ICP. ICP rat models were established by injecting 17α-ethinylestradiol (EE) subcutaneously from the thirteenth day of gestation for four days and then treated with EE (D group, n=5), resveratrol (R group, n=5), or ursodeoxycholic acid (UDCA; U group, n=5) from the seventeenth to the twentieth day of gestation. Fecal samples were analyzed with 16S ribosomal RNA (rRNA) sequencing. In results: the gut microbiota of pregnant rats was characterized with reduced alpha diversity (Chao1 index), and significant variation in the microbiota structure (ANOSIM) was also observed after being treated with EE. The richness of four phyla and ten genera was upregulated, and five phyla and ten genera were downregulated by EE treatment. The dysbiosis of Bilophila, Ruminococcus, and Actinobacteria caused by EE treatment was reversed by resveratrol administration. There was a correlation between total bile acid and alanine aminotransferase in ICP rats. The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis results suggested that the secondary bile acid biosynthesis was decreased, and the alanine, aspartate, and glutamate metabolism was increased after being treated with EE in pregnant rats. In conclusion, EE treatment could lead to gut microbiome dysbiosis and bile acid metabolism dysregulation in pregnant rats. Resveratrol could partially rescue gut microbiota dysbiosis and improve the biochemical characteristics caused by EE treatment.

Key words: *bile acids, gut microbiome, intrahepatic cholestasis of pregnancy, resveratrol, 17α-ethinylestradiol, alanine aminotransferase, Bacteroidetes, Firmicutes*

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disorder characterized by itching and elevated serum total bile acid (TBA) and/or alanine aminotransferase (ALT). It usually develops in the second and third trimesters. A serum TBA concentration higher than 10 μmol/L is considered the diagnostic criterion for ICP (1). The underlying mechanisms of ICP are still not fully understood. Estrogens have been proven to implicate the pathogenesis of ICP (2), recent studies have found that ICP is accompanied by a gut bacteria disorder (3).

There is a close relationship between gut bacteria and the metabolism of bile acids (4, 5). Dysbiosis of gut bacteria is associated with various diseases related to cholestasis, including primary biliary cholangitis and ICP (6, 7). Ursodeoxycholic acid (UDCA) improves gut microflora dysbiosis in patients with primary biliary cholangitis and ICP (8). It is associated with enrichment of the gut microbiota of Bacteroidetes and increases the ratio of Bacteroidetes to Firmicutes in ICP patients (9, 10). Previous studies suggested that the pathological process of cholestasis includes oxidative stress and inflammation (11, 12). Resveratrol has been postulated to modulate the gut microbiota composition in cardiovascular diseases, obesity, and diabetic nephropathy (13, 14). Beside, resveratrol can modulate autophagy and induce apoptosis in cancer cell lines *in vitro* including human leukemic cells (14, 15), resveratrol and as one of the natural polyphenolic compounds, can be also effective in the prophylaxis and prevention osteoporosis (16). In atherosclerosis rats, resveratrol increased the abundance of Lactobacillus and Bifidobacterium, which further increased the bile salt hydrolase activity, thereby enhancing bile acid deconjugation and fecal excretion (17). Our previous study indicated that orally administered resveratrol has anti-inflammatory effects and can reduce serum TBA in ICP rats (18). However, the effect of resveratrol on the gut microbiota in ICP rats remains unclear.

ICP rat model induced by estrogen is a classic animal model, it is accompanied by impairing transport mechanisms of bile acids in both basolateral and canalicular hepatocyte membranes (19, 20). In the present study, we established an ICP rat model (18) by injecting 17α-ethinylestradiol (EE) subcutaneously from the thirteenth to the sixteenth day of gestation, and then the rats were treated with resveratrol or UDCA from the seventeenth to

the twentieth day of gestation. The fecal bacterial community of all subjects was investigated with 16S ribosomal RNA (rRNA) gene sequencing. This study reveals the dysbiosis of gut microbiota and the effects of resveratrol on gut microbiota in ICP rats. It is helpful to find potential therapeutic strategies for ICP.

MATERIALS AND METHODS

Study design and samples

Adult Sprague Dawley rats weighing 200–280 g were purchased from the Chengdu Dossy Experimental Animals Co., Ltd. (Sichuan, China). All rats had free access to food and water.

All pregnant rats (n=20) were randomly divided into the control group (n=5) and the treated group (n=15) injected with EE (E4876, U.S.) subcutaneously from the thirteenth day of gestation for four days. The pregnant rats treated on the thirteenth day were considered the EE13 group, and the pregnant rats treated on the seventeenth day (n=15) were considered the EE17 group (the appearance of the vaginal plug of the female rats was taken as the first day of pregnancy). Then, rats in the EE17 group were divided into the ICP: D subgroup (for disease, n=5), R subgroup (for resveratrol, R5010, U.S., n=5), and U subgroup, (UDCA, U5127, U.S., n=5) The procedure is shown in *Fig. 1*.

1. Control group: the rats were injected with 5 mg/(kg/d) olive oil subcutaneously from the thirteenth day of gestation for eight days, followed by oral administration of 8 ml/(kg/d) 2% dimethylsulfoxide (DMSO) (2 ml DMSO + 98 ml normal saline (NS)) administered from the seventeenth day of gestation for four days.
2. D group: the rats were given subcutaneous injections of EE (5 mg/(kg/d), 1 mg EE dissolved in 1 ml olive oil) from the thirteenth to the twentieth day of gestation, followed by oral administration of 8 ml/(kg/d) 2% DMSO from the seventeenth to the twentieth days of gestation.
3. R group: the rats were injected with EE 5 mg/(kg/d) subcutaneously from the thirteenth to the twentieth day of gestation, followed by oral administration of resveratrol (RSV) (60 mg/(kg/d) (750 mg RSV+2 ml DMSO+98 ml NS)) from the seventeenth to the twentieth days of gestation.
4. U group: the rats were injected with EE (5 mg/(kg/d)) subcutaneously from the thirteenth to the twentieth days of gestation, followed by oral administration of UDCA (25 mg/(kg/d) (312.5 mg UDCA+2 ml DMSO+98 ml NS)) from the seventeenth to the twentieth days of gestation.

Caudal vein blood and fecal samples of the fasting rats were collected between 9:00 and 11:00 am before other management was taken on the thirteenth, seventeenth, and twenty-first days of gestation. The serum TBA and ALT were measured using the enzymatic cycling assay and kinetic rate method. Gut microbiota was analyzed by 16S rRNA gene sequencing. The animals were sacrificed on the twenty-first day of gestation.

Ethics approval and consent to participate

Principles of Laboratory Animal Care' (NIH Publication Vol 25, No. 28 revised 1996; http://grants.nih.gov/grants/guide/notice-files/not96-208. html) were followed, as well as specific national laws (*e.g.* the current version of the German Law on the Protection of Animals) where applicable.

The animal study was approved by the Animal Ethics Committee of Chongqing Medical University.

DNA extraction, 16S rRNA sequencing, and quality control

DNA was extracted from the fecal samples using a viral RNA and DNA extraction kit (Surbiopure Biotechnology, Guangzhou, China). The concentration and purity were measured using the NanoDrop One (ThermoFisher Sci., Waltham, MA, USA). The V3–V4 regions of the bacterial 16S rRNA genes were amplified using the specific primers 314F (5'-barcode-CCTACGGGNGGCWGCAG-3') and 806R (5'-barcode-GGACTACHVGGGTWTCTAAT-3'). The primers were synthesized by Invitrogen (Invitrogen, Carlsbad, CA, USA). Polymerase chain reaction (PCR) products were blended in equidensity ratios according to the GeneTools Analysis Software (Version 4.03.05.0, Syngene, Bengaluru, India). Then, the mixture of PCR products was purified with an

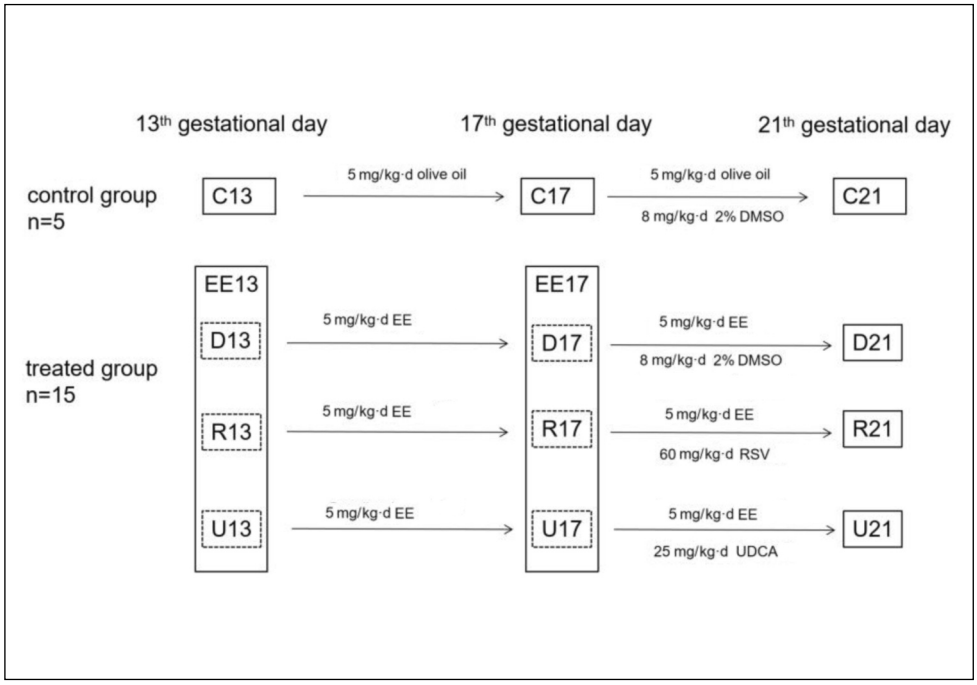

Fig. 1. The experimental strategies on pregnant rats. In the control group, five rats were treated with olive oil for eight days. In the treated group, 15 rats were treated with EE for four days; the rats on the thirteenth day of gestation were defined as the EE13 group, and rats on the seventeenth day of gestation were defined as the EE17 group. The rats on the seventeenth day of gestation were divided into the intrahepatic cholestasis of pregnancy (D17), resveratrol (R17), and UDCA groups (U17), then treated with EE, resveratrol, or UDCA for four days, respectively. *Abbreviations:* EE, 17α-ethinylestradiol; U/UDCA, ursodeoxycholic acid; R, resveratrol; D, disease, stands for intrahepatic cholestasis of pregnancy.

EZNA Gel Extraction Kit (Omega Bio-Tek, Norcross, GA, USA). Each project selects the appropriate primers for amplification. When the final primer sequence is not known, it can be viewed in the mapping file of the analysis result package.

Sequencing libraries were generated using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations. The library quality was assessed on the Qubit 2.0 Fluorometer (ThermoFisher Sci., Waltham, MA, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Waldbronn, Germany). Finally, the library was sequenced on an Illumina HiSeq 2500 platform, and 250 bp paired-end reads were generated.

Gut microbiota analysis

Alpha diversity was applied by analyzing the complexity of species diversity for a sample through two indices (Chao1 and Shannon-e). All indices in our samples were calculated with QIIME (v 1.9.1) and displayed with the R software (v 2.15.3). The Chao1 index was chosen to identify the community richness,

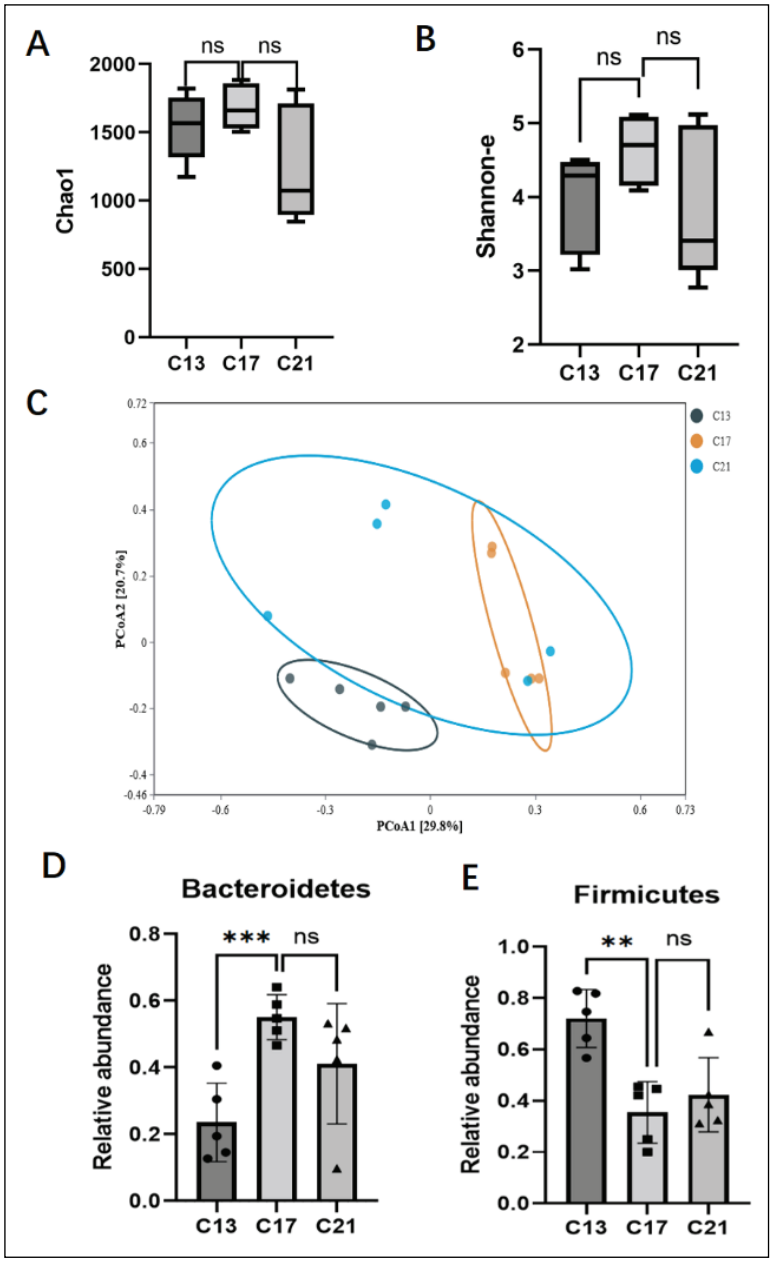

Fig. 2. The gut microbiota structure and composition of pregnant rats in the control group. (A, B): Chao1 and Shannon-e alpha diversity indexes in pregnant rats in the control group. (C): The PCoA plots of the Bray-Curtis distances in the control group. Dots in gray, orange, and blue indicate the thirteenth, seventeenth, and twenty-first days of gestation, respectively. The p-value was tested with ANOSIM (C13 vs. C17, p=0.013, and C17 vs. C21, p=0.216). (D, E): The relative abundance of Bacteroidetes and Firmicutes between the C13 and C17 groups. **p<0.01; ***p<0.001; ns - not significant.
Abbreviations: C13, the thirteenth day of gestation in the control group; C17, the seventeenth day of gestation in the control group; C21, the twenty-first day of gestation in the control group; PCoA, principal coordinate analysis; ANOSIM, analysis of similarity.

Table 1. Biochemical characteristics in EE13 group and EE17 group.

Groups	**n**	**TBA** (μmol/L)	**ALT** (U/L)
EE13	15	9.74±5.99	7.59±7.54
EE17	15	42.55±36.88**	51.18±18.88***

and the Shannon-e index was used to determine the community diversity. A beta diversity analysis was used to evaluate the differences in samples of species complexity. The Bray-Curtis index was calculated by QIIME software. A Principal Coordinate Analysis (PCoA) was performed to get principal coordinates and visualization from complex, multidimensional data. The PCoA analysis was displayed by QIIME 2 and ggplot2 packages in the R software. The non-parametric analysis of similarity (ANOSIM) was performed with the R software based on the otu_table_subsample to reveal the extent of differences between the groups and whether the differences were significant. Linear discriminant analysis Effect Size (LEfSe) analyses were used to find the biomarker of each group.

Statistical analysis

The statistical significance of the differences between the groups of rats was assessed using the R v 3.6.1 and GraphPad Prism software v 9.0 (GraphPad Software, San Diego, CA, USA). The differential abundances of species were performed with one-way analysis of variance (ANOVA) and non-parametric tests, including the Kruskal-Wallis rank sum test, Wilcoxon rank sum test, and Mann-Whitney U test. Data are presented as mean ±standard deviation (SD) for the indicated number of independently performed experiments, and p-values <0.05 were considered statistically significant. Correlations between the bacteria and liver parameters in the ICP rats were calculated using Spearman's rank correlation analysis with the R software.

RESULTS

Gut microbial community in pregnant rats

In the control group, fecal samples of the pregnant rats on the thirteenth, seventeenth, and twenty-first days of gestation were collected and analyzed with 16S rRNA to investigate the intestinal microbial community. The alpha diversity was assessed. The richness and diversity were indicated by the Chao1 and Shannon-e estimators, and no significant difference was found among the thirteenth, seventeenth, and twenty-first days of gestation (*Fig. 2A* and *2B*). Furthermore, a PCoA based on the Bray-Curtis distance was performed to assess the structural similarities in the control group. The PCoA results showed the bacterial structure on the thirteenth day of gestation was distinctly separated from the seventeenth day (ANOSIM, p=0.013), while no significant difference was found between the seventeenth and twenty-first days of gestation (ANOSIM, p=0.216) (*Fig. 2C*). At the phylum level, the relative abundance of Bacteroidetes increased, while Firmicutes decreased on the seventeenth day of gestation compared with the thirteenth day (*Fig. 2D* and *2E*, p<0.05). However, no significant difference was found between the seventeenth and twenty-first days of gestation in the control group.

Effects of 17α-ethinylestradiol on the biochemical characteristics and gut microbial community in pregnant rats

The serum levels of TBA and ALT in the EE17 group were significantly increased compared with the EE13 group (*Table 1*).

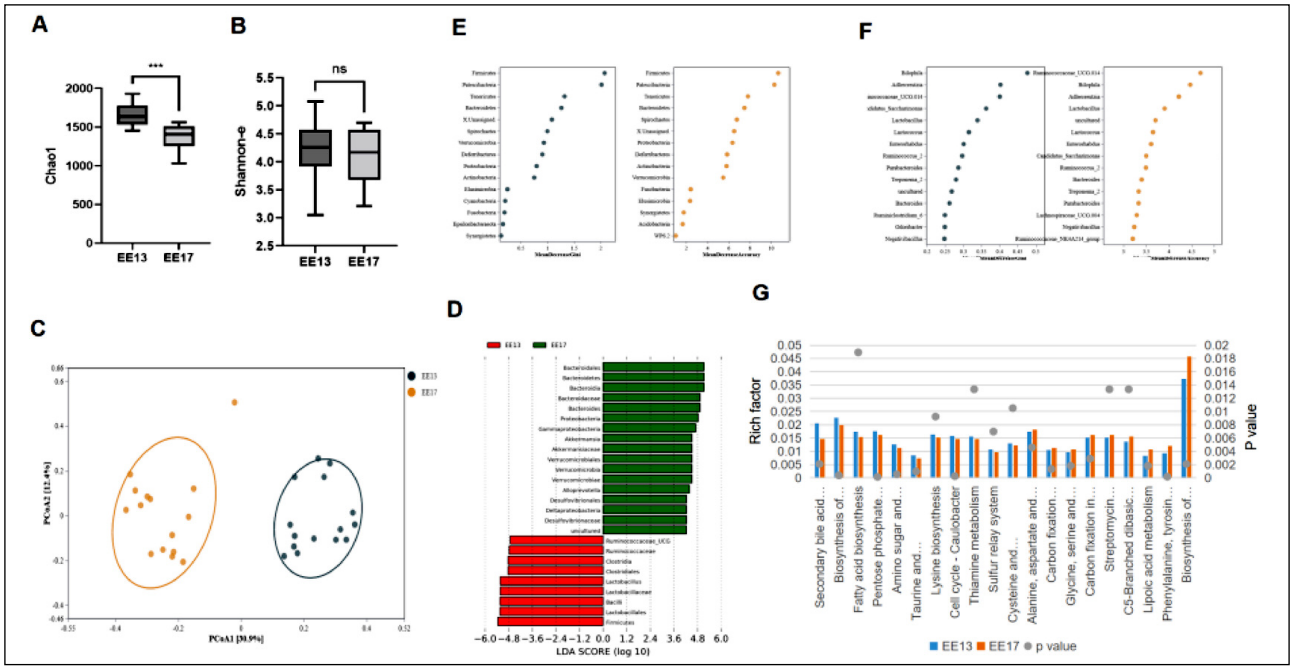

Fig. 3. The effects of EE on gut microbiota in pregnant rats. (A, B): The Chao1 and Shannon-e alpha diversity indexes in pregnant rats between the EE13 and EE17 groups. (C): The PCoA of the fecal microbiome in pregnant rats between the EE13 and EE17 groups. (D): The relative abundances of dominant bacterial from phylum to genus in bacterial communities in the EE13 and EE17 groups were analyzed using LEfSe (LDA>4). (E, F): The random forest indicates the most discriminating bacteria in descending order of importance at the phylum and genus levels. (G): The KEGG pathway enrichment analysis of metabolites was identified in this study. The metabolites identified were subjected to a pathway enrichment analysis using the KEGG database. Only significantly enriched KEGG functional categories are depicted according to their p-values (p<0.05). The p-values were calculated by the Mann-Whitney U test. ***p<0.001 and ns - not significant.

Abbreviations: EE, 17α-ethinylestradiol; EE13, the thirteenth day of gestation in the EE group; EE17, the seventeenth day of gestation in the EE group; LEfSe, linear discriminant analysis Effect Size; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCoA, principal coordinate analysis.

Then, the gut bacterial community diversity in the EE13 and EE17 groups were analyzed. The Chao1 index of the EE17 group was significantly reduced compared with the EE13 group (*Fig. 3A*), and the Shannon-e index showed no significant difference between the two groups (*Fig. 3B*). Furthermore, PCoA demonstrated that the bacterial structure in the EE17 group was distinctly separated from the EE13 group (*Fig. 3C*). Then we analyzed the abundance of bacteria in the EE13 and EE17 groups. At the phylum level, the abundance of four bacterial phyla was significantly increased, while the other five bacterial phyla decreased considerably in the EE17 group compared with the EE13 group (*Fig. 4A*). At the genus level, the abundance of ten bacterial genera was significantly increased, while the other ten bacterial genera decreased considerably in the EE17 group compared with the EE13 group (*Fig. 4B*). The results suggested that EE reduced the bacterial richness and altered the bacterial structure in the feces of pregnant rats.

A correlation analysis was also performed between the gut bacteria and biochemical characteristics (TBA and ALT) in the EE13 and EE17 groups. The results showed strong correlations (correlation coefficient, R>0.5) between serum biochemical characteristics and specific bacteria based on Pearson correlation coefficients:

1. At the phylum level, Firmicutes, Spirochaetes, Patescibacteria, and Tenericutes were negatively associated with TBA and ALT, while Proteobacteria, Bacteroidetes, and Verrucomicrobia were positively associated with TBA and ALT (*Fig. 4C*).

2. At the genus level, Akkermansia, Parabacteroides, Bacteroides, and Butyricimonas were positively associated with TBA and ALT, while Ruminococcaceae_UCG-005, Turicibacter, Christensenellaceae_R-7_group, Ruminococcaceae_NK4A214 _group, Lactobacillus, Ruminococcaceae_UCG-014, and Ruminiclostridium_6 were negatively associated with TBA and ALT, and Ruminococcus_1 was negatively associated with ALT (*Fig. 4D*).

The LEfSe of bacteria abundance was applied between the EE13 and EE17 groups, and the most significant differences (LDA score >4.0) in the gut bacteria (from the phylum to genus levels) in the two groups were identified (*Fig. 3D*), which can be considered as the biomarkers in normal and ICP rats. We used random forests for classification analysis to determine the different main bacteria at the phylum and genus levels between the EE13 and EE17 groups. The score of Mean Decrease Accuracy or Mean Decrease Gini reflects the degree of importance (*Fig. 3E* and *3F*).

Differential metabolites with known Kyoto Encyclopedia of Genes and Genomes (KEGG) identifications were used for enrichment analysis of the KEGG pathway to elucidate the pathways affected by EE. The differences of 20 pathways were identified between the two groups, and eight of them (including alanine, aspartate, and glutamate metabolism) were significantly more abundant in the EE17 group than in the EE13 group, while 12 of the pathways (including secondary bile acid biosynthesis) were downregulated by EE treatment (*Fig. 3G*). The KEGG

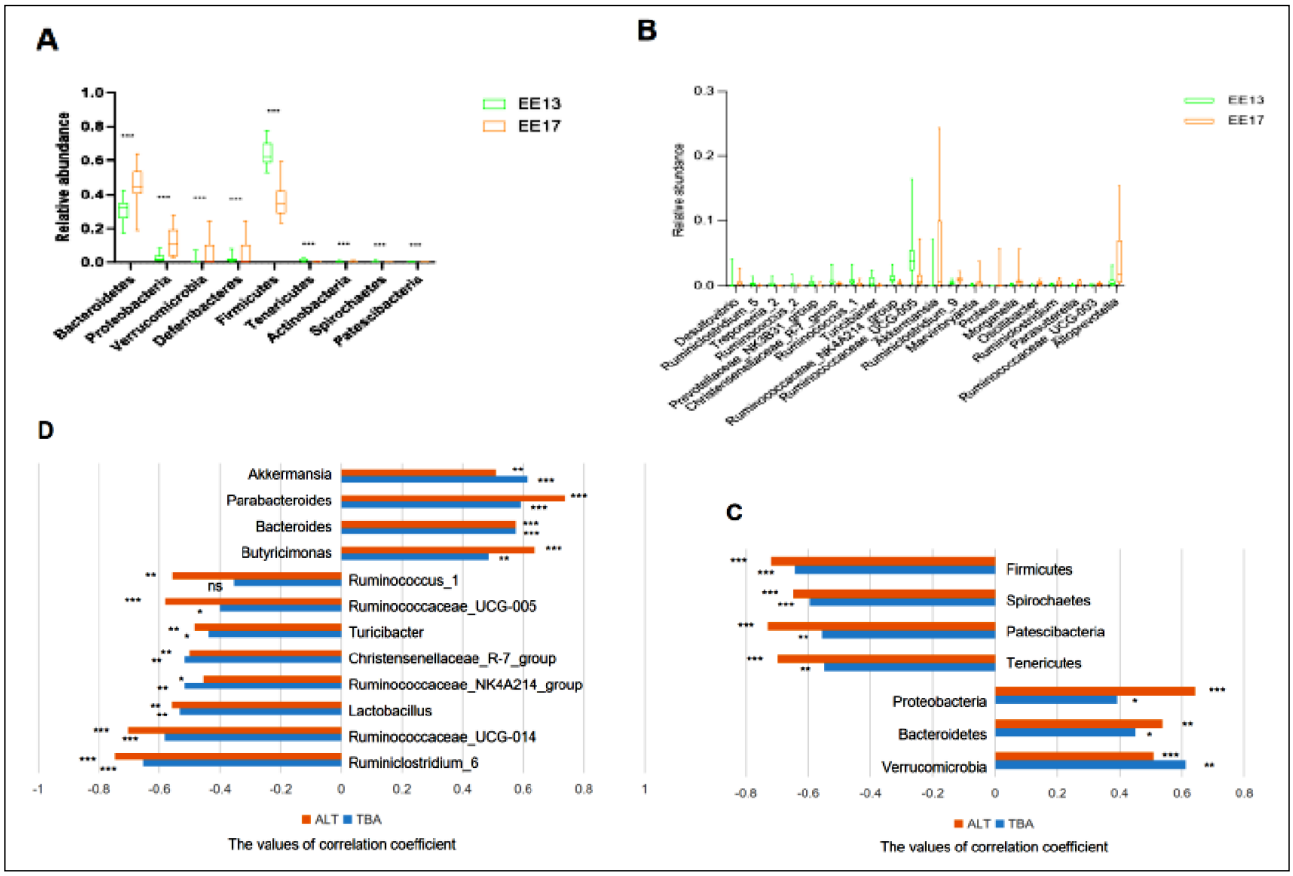

Fig. 4. Effects of EE on gut microbiota in pregnant rats. (A, B): The relative abundance of differential bacteria between the EE13 and EE17 groups at the phylum and genus levels. (C, D): The correlations between bacteria and clinical parameters (total bile acids and alanine aminotransferase) in the EE13 and EE17 groups. Pearson correlation coefficients were tested between bacteria and two biochemical parameters. *p<0.05; **p<0.01; ***p<0.001; ns - not significant.

Abbreviations: EE, 17α-ethinylestradiol; EE13, the thirteenth day of gestation in the EE group; EE17, the seventeenth day of gestation in the EE group.

analysis showed EE treatment altered the metabolism of ALT, AST, and bile acid profiles in pregnant rats.

Effects of resveratrol and ursodeoxycholic acid on gut microbial structure in pregnant rats with cholestasis

The Chao1 and Shannon-e indexes in the R21 group were significantly downregulated by resveratrol treatment compared with the R17 group, and the PCoA of the 16S rRNA sequence data of fecal contents showed a clear separation based on community structure between the seventeenth and twenty-first days of gestation in the R group. These results indicated that resveratrol treatment reduced the richness and diversity of gut bacteria and also altered the bacterial community structure in ICP rats. The LEfSe analysis of bacteria abundance was applied between the R17 and R21 groups, and the most significant differences (LDA score >4.0) in the bacteria (from the phylum to genus levels) were identified. At the phylum level, the relative abundance of Actinobacteria, Synergistetes, and Chloroflexi were higher, while Tenericutes were lower in the R21 group than in the R17 group. At the genus level, the relative abundance of five bacterial genera was significantly increased, and eleven bacterial genera were decreased considerably in the R21 group compared with the R17 group. Interestingly, EE treatment decreased the abundance of Actinobacteria and increased the abundance of Ruminiclostridium and Bilophila, which can be reversed by resveratrol treatment. Compared with the R17 group, the serum levels of TBA and ALT were significantly reduced in the R21 group. This means that resveratrol treatment is helpful to ameliorate the dysbiosis of gut microbiota in ICP rats.

As one of the primary drugs for the treatment of ICP, UDCA has been investigated for regulating gut microbiota in ICP rats. No significant difference was found between the U17 and U21 groups in alpha and beta diversity or TBA and ALT serum levels.

DISCUSSION

Increasing evidence supports the pathogenic role of gut microbiota in diseases associated with cholestasis. However, the underlying mechanism of gut microbiota in ICP remains unknown. The present study demonstrated that gut microbiota dysbiosis in the ICP rat model correlates with elevated serum biochemical indicators (TBA and ALT). The study also suggested that EE treatment decreased the biosynthesis of

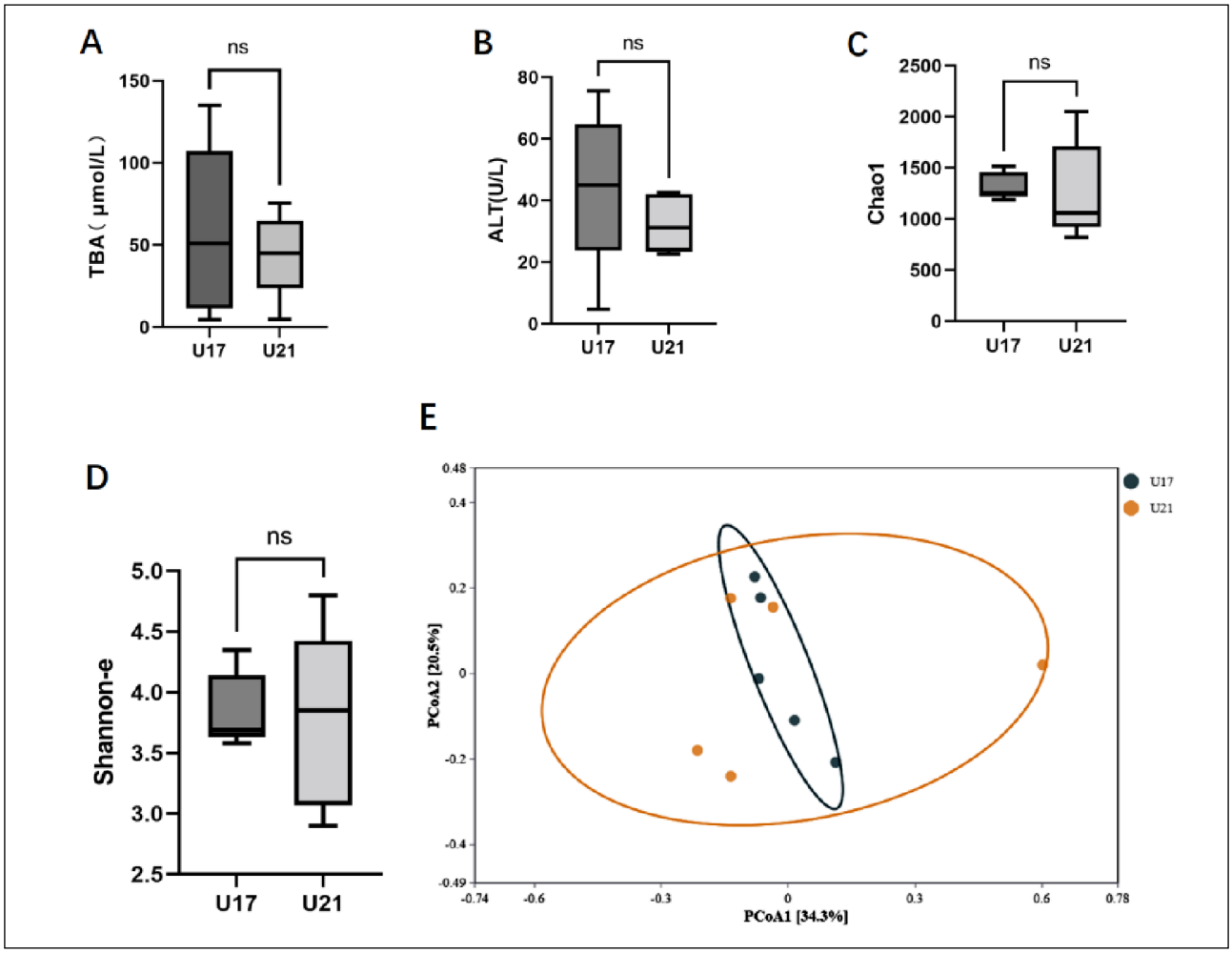

Fig. 5. The comparison of TBA (A) and ALT (B) in U17 group and U21 group suggested no significant difference ($p>0.05$). Alpha diversity was applied by analyzing the complexity of species diversity for a sample through Chao1 and Shannon-e (C, D). A beta diversity analysis was used to evaluate the differences in samples of species complexity (E).
Abbreviations: TBA, total bile acid, ALT, alanine aminotransferase, U17 and U21, the 17th and 21th day of gestation in UDCA group. ns - $p>0.05$.

secondary bile acid and upregulated the metabolism of alanine, aspartate, and glutamate in ICP rats. In addition, the study showed for the first time that resveratrol could reverse the dysbiosis of Actinobacteria phylum and Ruminiclostridium and Bilophila genera induced by EE treatment in ICP rats.

Changes in intestinal flora during pregnancy are unclear. In 2012, Koren *et al.* (21) found gut microbiota changed dramatically from the first to third trimesters, the α-diversity reduced, and β-diversity increased in late pregnancy compared with early pregnancy. In our study, PCoA showed that the gut microbiota structure in rats on the thirteenth gestational day was distinctly separate from the seventeenth, and it remained stable between the seventeenth and twenty-first days of gestation. The relative abundance of Bacteroides increased, and Firmicutes decreased on the seventeenth day of gestation compared with the thirteenth day. However, DiGiulio *et al.* (22) found that pregnancy progression is not associated with a dramatic remodeling of the diversity and composition of a woman's indigenous microbiota. In the presented study, we found the community richness(chao 1 index) and community diversity (shannon-e index) remained stable among the C13, C17and C21 group. on the other hand, we found the relative abundance of Bacteroidetes increased and Firmicutes decreased in the C13 group compared with C17 group.

Gut bacteria regulate the formation of bile acid profiles (23, 24) and Bacteroidetes deconjugate bile acids by secreting bile salt hydrolase (BSH). Verrucomicrobia can transform primary bile acids to secondary bile acids (9, 25). Ovadia *et al.* (23) found a high abundance of Bacteroidetes and a high ratio of Bacteroidetes to Firmicutes are associated with secreting BSH and aryl sulfate, which helps to degrade conjunction bile acids and their products taurine and glycine, that might result in decreasing conjunction, secondary, and sulfated bile acids. We found that the abundance of Bacteroidetes increased both in the control and EE groups on the seventeenth day of gestation compared with the thirteenth day, which may be a self-protective regulation. The present study identified an interaction between gut bacteria and the bile acids profile. The KEGG pathway analysis demonstrated that EE treatment decreased secondary bile acid biosynthesis. This is consistent with previous reports, which showed that the primary bile acid biosynthesis pathways were enriched in the ICP patients compared with healthy controls (26). The ICP patients are associated with a high abundance of taurocholic acid and a low abundance of lithocholic acid (LCA) and UDCA (27, 28). Studies have shown that chenodeoxycholic and cholic acids or their combination with taurine could activate Farnesoid X receptor and fibroblast growth factor 15/19, which further modulate the enterohepatic circulation of bile acids (8).

We further analyzed the specific bacteria which play important roles in the metabolism of bile acids. Bilophila, enriched in the feces of patients with ICP (26), plays a vital role in bile acid metabolism. The present study observed that the abundance of Bilophila is upregulated by EE treatment. We also observed that resveratrol could downregulate the abundance of Bilophila in ICP rats. This is similar to previous studies, which showed that resveratrol could reduce the abundance of Bilophila and benefits subjects in insulin-resistant rats (29, 30). So Bilophila might be a probiotic in the treatment of ICP. Ruminococcus is important in bile acids biotransformation and glucose homeostasis (31). Actinobacteria take part in the metabolism of bile acids through stereospecific hydroxylation of LCA at position 7β (32). The present study demonstrated that resveratrol could reverse the dysbiosis of Bilophila, Ruminococcus, and Actinobacteria caused by EE treatment and improve serum TBA and ALT. Therefore, we propose that resveratrol may be a new drug targeting gut microbiota to treat ICP.

Our study has several limitations. Firstly, this study found no significant difference in bacteria in the U21 group compared with the U17 group after UDCA treatment, which may be related to the sample size of this subgroup (n=5). On the other hand, although the present study identified the correlation between ICP rats and intestinal bacteria, the causal relationship between them remains unknown, and the fecal microbiota transplantation experiments should be further performed in the future.

In conclusion: resveratrol treatment is a potential therapeutic intervention against EE-induced abnormal metabolism of bile acid, at least in part by improving gut microbiota. Our findings contributed to a better understanding of the effect of fecal microbiota dysbiosis in the occurrence of ICP and provided a new treatment strategy for it.

Authors' contributions: Conception and design of the research: Zhizun Li, Zhengai Xiong,Yong Shao; acquisition of data: Zhizun Li, Lei Lei; analysis and interpretation of the data: Zhizun Li, Li Ling, Yong Shao; statistical analysis: Zhizun Li, Yang Liu; obtaining financing: Yong Shao, writing of the manuscript: Zhizun Li; critical revision of the manuscript for intellectual content: Zhengai Xiong, Li Ling, Yong Shao. All authors read and approved the final draft.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding: This work is funded by National Natural Sciences Foundation of China (No:81471473) and Chongqing Health Planning Commission project (No. 2019ZDXM055).

Conflict of interests: None declared.

REFERENCES

1. Geenes V, Chappell LC, Seed PT, Steer PJ, Knight M, Williamson C. Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: a prospective population-based case-control study. *Hepatology* 2014; 59: 1482-1491.
2. Yamamoto Y, Moore R, Hess HA, *et al.* Estrogen receptor alpha mediates 17alpha-ethynylestradiol causing hepatotoxicity. *J Biol Chem* 2006; 281: 16625-16631.
3. Zhan Q, Qi X, Weng R, *et al.* Alterations of the human gut microbiota in intrahepatic cholestasis of pregnancy. *Front Cell Infect Microbiol* 2021; 11: 635680. doi: 10.3389/fcimb.2021.635680
4. Yang ZH, Liu F, Zhu XR, Suo FY, Jia ZJ, Yao SK. Altered profiles of fecal bile acids correlate with gut microbiota and inflammatory responses in patients with ulcerative colitis. *World J Gastroenterol* 2021; 27: 3609-3629.
5. Wahlstrom A, Sayin SI, Marschall HU, Backhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab* 2016; 24: 41-50.
6. Chen W, Wei Y, Xiong A, *et al.* Comprehensive analysis of serum and fecal bile acid profiles and interaction with gut microbiota in primary biliary cholangitis. *Clin Rev Allergy Immunol* 2020; 58: 25-38.
7. Li R, Chen X, Liu Z, *et al.* Characterization of gut microbiota associated with clinical parameters in intrahepatic cholestasis of pregnancy. *BMC Gastroenterol* 2020; 20: 395. doi: 10.1186/s12876-020-01510-w
8. Tang R, Wei Y, Li Y, *et al.* Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut* 2018; 67: 534-541.

9. Ovadia C, Perdones-Montero A, Fan HM, *et al.* Ursodeoxycholic acid enriches intestinal bile salt hydrolase-expressing Bacteroidetes in cholestatic pregnancy. *Sci Rep* 2020; 10: 3895. doi: 10.1038/s41598-020-60821-w
10. Kong X, Kong Y, Zhang F, Wang T, Yan J. Evaluating the effectiveness and safety of ursodeoxycholic acid in treatment of intrahepatic cholestasis of pregnancy: a meta-analysis (a prisma-compliant study): *Medicine* (*Baltimore*) 2016; 95: e4949. doi: 10.1097/MD.0000000000004949
11. Hou HP, Zhang G-P, Han Li H, *et al.* Mitochondria are main targets of time/dose-dependent oxidative damage-based hepatotoxicity caused by rhizoma dioscoreae bulbiferae in mice. *World J Tradit Chin Med* 2020; 6: 461-468.
12. Zheng YY, Xiao Wang X, Si JT, *et al.* Randomized clinical trials of traditional chinese medicines for treating ulcerative colitis: a scoping review. *World J Tradit Chin Med* 2021; 7: 326-331.
13. Bird JK, Raederstorff D, Weber P, Steinert RE. Cardiovascular and antiobesity effects of resveratrol mediated through the gut microbiota. *Adv Nutr* 2017; 8: 839-849.
14. Siedlecka-Kroplewska K, Wozniak M, Kmiec Z. The wine polyphenol resveratrol modulates autophagy and induces apoptosis in MOLT-4 and HL-60 human leukemia cells. *J Physiol Pharmacol* 2019; 70: 825-838.
15. Du QQ, Tang M, Huang LL, *et al.* The antitumor activity and mechanism of MCL3 in G422 glioblastoma. *World J Tradit Chin Med* 2020; 6: 353-361.
16. Cai TT, Ye XL, Li RR, *et al.* Resveratrol modulates the gut microbiota and inflammation to protect against diabetic nephropathy in mice. *Front Pharmacol* 2020; 11: 1249. doi: 10.3389/fphar.2020.01249
17. Chen ML, Yi L, Zhang Y, *et al.* Resveratrol attenuates trimethylamine-n-oxide (TMAO)-induced atherosclerosis by regulating TMAO synthesis and bile acid metabolism via remodeling of the gut microbiota. *mBio* 2016; 7: e02210-e02215.
18. Liao E, Li Z, Shao Y. Resveratrol regulates the silent information regulator 1-nuclear factor-kappaB signaling pathway in intrahepatic cholestasis of pregnancy. *Hepatol Res* 2018; 48: 1031-1044.
19. Reyes H, Simon FR. Intrahepatic cholestasis of pregnancy: an estrogen-related disease. *Semin Liver Dis* 1993; 13: 289-301.
20. Rodriguez-Garay EA. Cholestasis: human disease and experimental animal models. *Ann Hepatol* 2003; 2: 150-158.
21. Koren O, Goodrich JK, Cullender TC, *et al.* Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012; 150: 470-480.
22. DiGiulio DB, Callahan BJ, McMurdie PJ, *et al.* Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci USA* 2015; 112: 11060-11065.
23. Ovadia C, Perdones-Montero A, Spagou K, *et al.* Enhanced microbial bile acid deconjugation and impaired ileal uptake in pregnancy repress intestinal regulation of bile acid synthesis. *Hepatology* 2019; 70: 276-293.
24. Sabino J, Vieira-Silva S, Machiels K, *et al.* Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. *Gut* 2016; 65: 1681-1689.
25. Nichenametla SN, Mattocks DAL, Midya V, Shneyder J. Differential effects of sulfur amino acid-restricted and low-calorie diets on gut microbiome profile and bile acid composition in male C57BL6/J mice. *J Gerontol A Biol Sci Med Sci* 2021; 76: 1922-1929.
26. Li GH, Huang SJ, Li X, Liu XS, Du QL. Response of gut microbiota to serum metabolome changes in intrahepatic cholestasis of pregnant patients. *World J Gastroenterol* 2020; 26: 7338-7351.
27. Tribe RM, Dann AT, Kenyon AP, Seed P, Shennan AH, Mallet A. Longitudinal profiles of 15 serum bile acids in patients with intrahepatic cholestasis of pregnancy. *Am J Gastroenterol* 2010; 105: 585-95.
28. Manna LB, Ovadia C, Lovgren-Sandblom A, *et al.* Enzymatic quantification of total serum bile acids as a monitoring strategy for women with intrahepatic cholestasis of pregnancy receiving ursodeoxycholic acid treatment: a cohort study. *BJOG* 2019; 126: 1633-1640.
29. Li F, Han Y, Cai X, *et al.* Dietary resveratrol attenuated colitis and modulated gut microbiota in dextran sulfate sodium-treated mice. *Food Funct* 2020; 11: 1063-1073.
30. Chen K, Zhao H, Shu L, *et al.* Effect of resveratrol on intestinal tight junction proteins and the gut microbiome in high-fat diet-fed insulin resistant mice. *Int J Food Sci Nutr* 2020; 71: 965-978.
31. Lin S, Yang X, Long Y, *et al.* Dietary supplementation with Lactobacillus plantarum modified gut microbiota, bile acid profile and glucose homoeostasis in weaning piglets. *Br J Nutr* 2020; 124: 797-808.
32. Kollerov VV, Monti D, Deshcherevskaya NO, *et al.* Hydroxylation of lithocholic acid by selected actinobacteria and filamentous fungi. *Steroids* 2013; 78: 370-378.

Received: March 9, 2022
Accepted: April 30, 2022

Authors' address: Dr. Zhengai Xiong, Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Chongqing Medical University, No. 76 Linjiang Road, Yuzhong District, Chongqing 400010, China.
E-mail: xiongzhengaixzaj@126.com

Dr. Yong Shao, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing 400042, China.
E-mail: shaoyongsy8@126.com