

妊娠期高血压病患者血清 DNMT1 mRNA 和 LncRNA UCA1 水平表达与妊娠结局的关系

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摘要: **目的** 分析妊娠期高血压疾病 (hypertensive disorder complicating pregnancy, HDCP) 患者血清 DNA 甲基转移酶 1 (DNA methyltransferase 1, DNMT1) 信使 RNA (messenger RNA, mRNA)、长链非编码 RNA (long non-coding RNA, LncRNA) 尿路上皮癌胚抗原 1 (urothelial carcinoembryonic antigen 1, UCA1) 水平与妊娠结局的关系。**方法** 选取 2021 年 3 月~2023 年 8 月在邯郸市妇幼保健院诊治的 HDCP 患者 195 例为病例组、健康妊娠孕妇 195 例为对照组。收集所有孕妇临床资料并检测分娩前 1 天生化指标; 荧光定量 PCR 法检测血清 DNMT1 mRNA, LncRNA UCA1 水平; 根据病情将病例组分为妊娠期高血压 (pregnancy induced hypertension, PIH) 组、轻度子痫前期 (preeclampsia, PE) 组、重度 PE 组; 根据 HDCP 患者分娩时不良妊娠结局情况分为妊娠结局良好组和妊娠结局不良组; 比较对照组和病例组临床资料和生化指标、血清 DNMT1 mRNA, LncRNA UCA1 水平; 比较不同严重程度 HDCP 患者血清 DNMT1 mRNA 和 LncRNA UCA1 水平; 比较不同妊娠结局 HDCP 患者临床资料和生化指标、血清 DNMT1 mRNA 和 LncRNA UCA1 水平; 分析 HDCP 患者血清 DNMT1 mRNA 与 LncRNA UCA1 的相关性, 影响 HDCP 患者妊娠结局的因素, 血清 DNMT1 mRNA 和 LncRNA UCA1 对 HDCP 患者发生不良妊娠结局的预测价值。**结果** 与对照组比较, 病例组收缩压、舒张压、白细胞计数水平明显升高, 血清 DNMT1 mRNA (0.72 ± 0.18 vs 1.00 ± 0.04), LncRNA UCA1 (0.61 ± 0.16 vs 1.00 ± 0.02) 水平明显降低, 差异具有统计学意义 ($t=40.651, 32.595, 7.837, 21.205, 33.775$, 均 $P < 0.001$); PIH 组、轻度 PE 组、重度 PE 组血清 DNMT1 mRNA ($0.85 \pm 0.20, 0.74 \pm 0.18, 0.50 \pm 0.15$), LncRNA UCA1 ($0.77 \pm 0.18, 0.58 \pm 0.16, 0.43 \pm 0.13$) 水平依次降低, 差异具有统计学意义 ($F=52.687, 64.030$, 均 $P < 0.001$); HDCP 患者血清 DNMT1 mRNA 与 LncRNA UCA1 呈正相关 ($r=0.582, P < 0.001$); 与妊娠结局良好组比较, 妊娠结局不良组 HDCP 严重程度较高, 收缩压、舒张压、白细胞计数水平明显升高, 血清 DNMT1 mRNA (0.80 ± 0.20 vs 0.59 ± 0.15), LncRNA UCA1 (0.72 ± 0.17 vs 0.43 ± 0.14) 水平明显降低, 差异具有统计学意义 ($\chi^2=18.386, t=2.615 \sim 12.290$, 均 $P < 0.05$); 重度 PE [OR (95%CI) = 1.708 (1.193~2.445)], 收缩压 [OR (95%CI) = 1.495 (1.090~2.049)], 舒张压 [OR (95%CI) = 1.621 (1.076~2.442)] 是影响 HDCP 患者发生不良妊娠结局的危险因素, DNMT1 mRNA [OR (95%CI) = 0.833 (0.725~0.957)], LncRNA UCA1 [OR (95%CI) = 0.796 (0.696~0.909)] 是影响 HDCP 患者发生不良妊娠结局的保护因素 (均 $P < 0.05$); DNMT1 mRNA 和 LncRNA UCA1 二者联合预测 HDCP 患者发生不良妊娠结局的曲线下面积 (area under curve, AUC) 大于 DNMT1 mRNA 及 LncRNA UCA1 单独预测的 AUC (0.926 vs $0.832, 0.844$), 差异具有统计学意义 ($Z=2.932, 2.345$, 均 $P < 0.05$)。**结论** HDCP 患者血清 DNMT1 mRNA 和 LncRNA UCA1 水平均较低, 与病情程度、妊娠结局相关, DNMT1 mRNA 联合 LncRNA UCA1 检测对不良妊娠结局有较佳预测效能。

关键词: 妊娠期高血压疾病; DNA 甲基转移酶 1 信使 RNA; 长链非编码 RNA 尿路上皮癌胚抗原 1; 妊娠结局

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Relationship between the Expression of Serum DNMT1 mRNA and LncRNA UCA1 Levels and Pregnancy Outcome in Patients with Hypertensive Disorder Complicating Pregnancy

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Abstract: Objective To analyze the relationship between the expression of serum DNA methyltransferase 1 (DNMT1) messenger RNA (mRNA) and long non-coding RNA (LncRNA) urothelial carcinoembryonic antigen 1 (UCA1) levels and

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pregnancy outcomes in patients with hypertensive disorder complicating pregnancy (HDCP). **Methods** A total of 195 HDCP patients treated in Handan Maternal and Child Health Hospital from March 2021 to August 2023 were selected as the case group, and 195 healthy pregnant women were regarded as the control group. Clinical data of all pregnant women were collected, and biochemical indicators were detected 1 day before delivery. Serum DNMT1 mRNA and LncRNA UCA1 levels were detected by fluorescence quantitative PCR. The case group was divided into pregnancy induced hypertension (PIH) group, mild preeclampsia (PE) group, and severe PE group based on their condition. HDCP patients were divided into good pregnancy outcome group and bad pregnancy outcome group according to the adverse pregnancy outcome at delivery. Clinical data, biochemical indexes, serum DNMT1 mRNA and LncRNA UCA1 levels were compared between control group and case group. The serum DNMT1 mRNA and LncRNA UCA1 levels of HDCP patients with different severity were compared. The clinical data, biochemical indexes, serum DNMT1 mRNA and LncRNA UCA1 levels of HDCP patients with different pregnancy outcomes were compared. The correlation between serum DNMT1 mRNA and LncRNA UCA1 in HDCP patients, factors affecting pregnancy outcomes in HDCP patients, and the predictive value of serum DNMT1 mRNA and LncRNA UCA1 in adverse pregnancy outcomes in HDCP patients were analyzed. **Results** Compared with control group, the levels of systolic blood pressure, diastolic blood pressure and white blood cell count in case group were increased, while the levels of serum DNMT1 mRNA (0.72 ± 0.18 vs 1.00 ± 0.04) and LncRNA UCA1 (0.61 ± 0.16 vs 1.00 ± 0.02) were decreased, and the differences were statistically significant ($t=40.651$, 32.595 , 7.837 , 21.205 , 33.775 , all $P<0.001$). Serum DNMT1 mRNA (0.85 ± 0.20 , 0.74 ± 0.18 , 0.50 ± 0.15) and LncRNA UCA1 (0.77 ± 0.18 , 0.58 ± 0.16 , 0.43 ± 0.13) levels in PIH group, mild PE group and severe PE group were decreased successively, and the differences were statistically significant ($F=52.687$, 64.030 , all $P<0.001$). Serum DNMT1 mRNA in HDCP patients was positively correlated with LncRNA UCA1 ($r=0.582$, $P<0.001$). Compared with good pregnancy outcome group, the severity of HDCP, systolic blood pressure, diastolic blood pressure and white blood cell count were increased in the bad pregnancy outcome group, and the serum DNMT1 mRNA (0.80 ± 0.20 vs 0.59 ± 0.15) and LncRNA UCA1 (0.72 ± 0.17 vs 0.43 ± 0.14) levels were decreased, and the differences were statistically significant ($\chi^2=18.386$, $t=2.615\sim 12.290$, all $P<0.05$). Severe PE [OR(95%CI)=1.708 (1.193~2.445)], systolic blood pressure [OR(95%CI)=1.495(1.090~2.049)] and diastolic blood pressure [OR(95%CI)=1.621 (1.076~2.442)] were risk factors for adverse pregnancy outcomes in HDCP patients, while DNMT1 mRNA [OR(95%CI)=0.833 (0.725~0.957)] and LncRNA UCA1 [OR(95%CI)=0.796 (0.696~0.909)] were protective factors for adverse pregnancy outcomes in HDCP patients (all $P<0.05$). The area under curve (AUC) predicted by the combination of DNMT1 mRNA and LncRNA UCA1 for adverse pregnancy outcomes in HDCP patients was greater than that predicted by DNMT1 mRNA alone and LncRNA UCA1 alone (0.926 vs 0.832 , 0.844), and the differences were statistically significant ($Z=2.932$, 2.345 , all $P<0.05$). **Conclusion** Both serum DNMT1 mRNA and LncRNA UCA1 levels are low in HDCP patients, which is related to the degree of disease and pregnancy outcome. DNMT1 mRNA combined with LncRNA UCA1 detection may have a better predictive effect on adverse pregnancy outcome.

Keywords: hypertensive disorder complicating pregnancy; DNA methyltransferase 1 messenger RNA; long non-coding RNA urothelial carcinoembryonic antigen 1; pregnancy outcome

妊娠期高血压疾病(hypertensive disorder complicating pregnancy, HDCP)是一种危及产妇健康及胎儿正常发育的病理妊娠,多发于妊娠中晚期,会增加产后出血等风险^[1]。据报道,HDCP与我国10%~16%的孕产妇死亡率相关^[2-3]。然而,仍缺乏可实现在孕中晚期疾病诊断的精准指标,故而开展HDCP临床研究并筛选妊娠结局标志物对转变妊娠结局尤为关键。DNA甲基化参与整个妊娠阶段,而早期配子异常甲基化同样会增加孕妇HDCP发生率。DNA甲基转移酶1(DNA methyltransferase 1, DNMT1)是人体中发现最早的基础性表达基因,维持DNA甲基化状态、强度的关键酶,参与细胞基本生理活动,其表达水平异常参与多种疾病发生^[4]。马晓莉等^[5]发现, DNMT1在一氧化氮合酶

抑制剂(nitric oxide synthase inhibitor, L-NAME)诱导的胎盘滋养细胞中表达水平减少。长链非编码RNA(long non-coding RNA, LncRNA)尿路上皮癌胚抗原1(urothelial carcinoembryonic antigen 1, UCA1)在肿瘤发生和调控关键因子途径中表达,研究表明LncRNA UCA1在胚胎、胎盘和胎儿组织发育过程中高度转录,贯穿整个胚胎发育过程,已证实LncRNA UCA1在子痫前期(preeclampsia, PE)胎盘组织中表达减少,通过靶向微小RNA(microRNA, miR)-182-5p对滋养细胞侵袭、转移有着促进作用^[6]。但HDCP患者血清DNMT1, LncRNA UCA1水平变化与妊娠结局的关系尚未明确。基于上述背景,本研究旨在探索DNMT1信使RNA(messenger RNA, mRNA), LncRNA UCA1

与 HDCP 患者妊娠结局的相关性,为 HDCP 病理机制解析做铺垫,并有助于增加良好妊娠结局。

1 材料与方法

1.1 研究对象 选取2021年3月~2023年8月在邯郸市妇幼保健院诊治的 HDCP 患者 195 例为病例组,其中年龄 22~36 (29.58 ± 5.24) 岁,产前体质质量指数 (body mass index, BMI) $22.54 \sim 29.23$ (25.81 ± 2.69) kg/m^2 ,分娩孕周 34~42 (37.45 ± 2.26) 周,分娩次数:1 次者 77 例, ≥ 2 次者 118 例;分娩方式:剖宫产 87 例,阴道分娩 108 例;收缩压 (分娩前 1 天) $138.15 \sim 164.32$ (152.18 ± 7.36) mmHg,舒张压 (分娩前 1 天) $92.54 \sim 116.08$ (103.95 ± 7.85) mmHg。根据病情将病例组分为妊娠期高血压 (pregnancy induced hypertension, PIH) 组 ($n=67$)、轻度 PE 组 ($n=81$) 和重度 PE 组 ($n=47$)。选取同期我院健康妊娠孕妇 195 例为对照组,年龄 21~37 (28.69 ± 5.70) 岁,产前 BMI 为 $22.38 \sim 29.16$ (25.33 ± 2.40) kg/m^2 ,分娩孕周 34~42 (37.91 ± 2.50) 周,分娩次数:1 次者 81 例, ≥ 2 次者 114 例;分娩方式:剖宫产 84 例,阴道分娩 111 例;收缩压 (分娩前 1 天) $105.27 \sim 130.09$ (119.25 ± 8.59) mmHg,舒张压 (分娩前 1 天) $64.13 \sim 88.93$ (78.43 ± 7.61) mmHg。纳入标准:①病例组符合 HDCP 诊断标准^[7];②单胎妊娠;③无酗酒、嗜烟史;④自然怀孕。排除标准:①滥用药物;②孕前已有高血压病史或一级亲属高血压家族史;③恶性肿瘤患者;④缺少病例资料,无详细病程记录;⑤并发免疫系统疾病、慢性肾脏病、糖尿病等基础疾病。与对照组比较,病例组收缩压、舒张压明显升高,差异具有统计学意义 ($t=40.651, 32.595$, 均 $P < 0.001$);年龄、产前 BMI,分娩孕周、分娩次数、分娩方式差异无统计学意义 ($t/\chi^2=1.605, 1.859, 1.906, 0.170, 0.094$, 均 $P > 0.05$)。病例组和对照组孕妇均签署知情同意书,本研究经医院伦理委员会批准。收集 HDCP 患者分娩时不良妊娠结局情况:胎膜早破、胎儿窘迫、早产、胎儿生长受限、产后出血、死胎、低体重儿等。根据分娩时不良妊娠结局进一步分为妊娠结局良好组 ($n=122$) 和妊娠结局不良组 ($n=73$),见表 1。与妊娠结局良好组比较,妊娠结局不良组 HDCP 严重程度较高。收缩压、舒张压明显升高,差异具有统计学意义 (均 $P < 0.05$),年龄、产前 BMI、分娩孕周、分娩次数、分娩方式差异无统计学意义 ($P > 0.05$)。

1.2 仪器与试剂 全自动血液分析仪 (BD-5180)、全自动生化分析仪 (Beckman AU640);荧光定量 PCR (qRT-PCR) (型号 eQ164CP, 苏州科铭生物公司);Trizol 试剂、一步法逆转录试剂盒、qRT-

PCR 试剂盒 (批号分别为 abs9331, abs60077, abs60088, 天津肽链生物公司)。

1.3 方法

1.3.1 生化指标检测:所有孕妇均在分娩前 1 天收集空腹外周静脉血 8 ml,分成两份,一份经抗凝后用全自动血液分析仪测定白细胞计数;另一份血液样本离心并分离血清,取部分血清,采用全自动生化分析仪检测总胆固醇、空腹血糖、三酰甘油。

表 1 不同妊娠结局 HDCP 患者临床资料比较

[$\bar{x} \pm s, n (\%)$]				
类别	结局良好组 ($n=122$)	结局不良组 ($n=73$)	t/χ^2	P
年龄 (岁)	29.16 ± 4.96	30.28 ± 5.71	1.441	0.151
产前 BMI (kg/m^2)	25.88 ± 2.82	25.69 ± 2.47	0.476	0.634
分娩孕周 (周)	37.24 ± 2.36	37.80 ± 2.09	1.672	0.096
分娩次数 (次)	1 49 (40.16)	28 (38.36)	0.062	0.803
	≥ 2 73 (59.84)	45 (61.64)		
分娩方式	剖宫产 51 (41.80)	36 (49.32)	1.043	0.307
	阴道分娩 71 (58.20)	37 (50.68)		
HDCP 严重程度	PIH 52 (42.62)	15 (20.55)	18.386	< 0.001
	轻度 PE 52 (42.62)	29 (39.73)		
	重度 PE 18 (14.75)	29 (39.73)		
收缩压 (mmHg)	147.72 ± 5.54	159.63 ± 10.39	10.433	< 0.001
舒张压 (mmHg)	102.55 ± 7.67	106.28 ± 8.14	3.212	0.002

1.3.2 qRT-PCR 检测血清 DNMT1 mRNA, LncRNA UCA1 水平:取剩余血清,利用 qRT-PCR 技术检测血清 DNMT1 mRNA 和 LncRNA UCA1 水平。方法如下:采用 Trizol 试剂抽提血清总 RNA,按照一步法逆转录试剂盒将总 RNA 反转录为 cDNA,采用 qRT-PCR 试剂盒以 cDNA 为模板扩增 DNMT1 mRNA, LncRNA UCA1, GAPDH (内参),引物序列见表 2。反应体系 26 μl ,其中包括:7 μl 的 qRT-PCR 试剂,正向引物、反向引物各 1 μl , 12 μl 的水,5 μl 的 cDNA。循环条件:93 $^{\circ}\text{C}$ 126 s;然后 90 $^{\circ}\text{C}$ 23 s, 64 $^{\circ}\text{C}$ 21 s, 73 $^{\circ}\text{C}$ 24 s, 共计 31 个循环。2 $^{-\Delta\Delta C_t}$ 法计算 DNMT1 mRNA, LncRNA UCA1 相对表达水平。

表 2 引物序列

基因	引物序列
DNMT1	正向: 5'-AGACTACGCCGAGATTCCGACTCGTAG-3' 反向: 5'-TTGGTGTGGCCTAGTAGTAGAAGGC-3'
LncRNA UCA1	正向: 5'-CTCTCTATCTCCCTTCACTGA-3' 反向: 5'-AGTTGGGTTGAGGTTCTGTCTCG-3'
GAPDH	正向: 5'-CACCATTGGCAATGACGGGTTTC-3' 反向: 5'-AGGTCTTTGCGGATGTCCACGT-3'

1.4 统计学分析 采用软件 SPSS 25.0 处理数据, 计量资料以均数 \pm 标准差 ($\bar{x} \pm s$) 表示, 两组间比较采用 t 检验; 单因素方差分析用于 PIH 组、轻度 PE 组、重度 PE 组三组间的比较, 进一步两两比较行 LSD- t 检验; 计数资料以 n (%) 表示, 采用 χ^2 检验; HDCP 患者血清 DNMT1 mRNA 与 LncRNA UCA1 的相关性用 Pearson 分析; HDCP 患者妊娠结局的影响因素用 Logistic 回归分析; 受试者工作特征 (ROC) 曲线评价血清 DNMT1 mRNA, LncRNA UCA1 对 HDCP 患者发生不良妊娠结局的预测价值, 曲线下面积 (area under curve, AUC) 比较行 Z 检验。 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 对照组、病例组生化指标和血清 DNMT1 mRNA, LncRNA UCA1 水平比较 见表 3。与对照组比较, 病例组白细胞计数水平明显升高, 血清 DNMT1 mRNA, LncRNA UCA1 水平均明显降低, 差异具有统计学意义 (均 $P < 0.05$); 总胆固醇、三酰甘油、空腹血糖差异无统计学意义 (均 $P > 0.05$)。

表 3 对照组和病例组生化指标和血清 DNMT1 mRNA, LncRNA UCA1 水平比较 ($\bar{x} \pm s$)

项 目	对照组 ($n=195$)	病例组 ($n=195$)	t	P
白细胞计数 ($\times 10^9/L$)	8.03 ± 1.34	9.49 ± 2.23	7.837	< 0.001
总胆固醇 (mmol/L)	4.11 ± 1.02	4.00 ± 0.96	1.097	0.273
三酰甘油 (mmol/L)	1.55 ± 0.29	1.60 ± 0.36	1.510	0.132
空腹血糖 (mmol/L)	5.05 ± 0.57	5.11 ± 0.63	0.986	0.325
DNMT1 mRNA	1.00 ± 0.04	0.72 ± 0.18	21.205	< 0.001
LncRNA UCA1	1.00 ± 0.02	0.61 ± 0.16	33.775	< 0.001

2.2 不同严重程度 HDCP 患者血清 DNMT1 mRNA, LncRNA UCA1 水平比较 见表 4。PIH 组、轻度 PE 组、重度 PE 组血清 DNMT1 mRNA, LncRNA UCA1 水平依次降低, 差异具有统计学意义 (均 $P < 0.05$)。

2.3 HDCP 患者血清 DNMT1 mRNA 与 LncRNA UCA1 的相关性分析 经 Pearson 分析发现, HDCP 患者血清 DNMT1 mRNA 与 LncRNA UCA1 呈正相

关 ($r=0.582, P < 0.001$)。

表 4 不同严重程度 HDCP 患者血清 DNMT1 mRNA, LncRNA UCA1 水平比较 ($\bar{x} \pm s$)

项 目	PIH 组 ($n=67$)	轻度 PE 组 ($n=81$)	重度 PE 组 ($n=47$)	F	P
DNMT1 mRNA	0.85 ± 0.20	0.74 ± 0.18^a	0.50 ± 0.15^{ab}	52.687	< 0.001
LncRNA UCA1	0.77 ± 0.18	0.58 ± 0.16^a	0.43 ± 0.13^{ab}	64.030	< 0.001

注: ^a与 PIH 组比较, $t=3.519, 6.795, 10.155, 11.075$, 均 $P < 0.05$; ^b与轻度 PE 组比较, $t=7.715, 5.463$, 均 $P < 0.05$ 。

2.4 不同妊娠结局 HDCP 患者生化指标和血清 DNMT1 mRNA, LncRNA UCA1 水平比较 见表 5。与妊娠结局良好组比较, 妊娠结局不良组白细胞计数水平明显升高, 血清 DNMT1 mRNA, LncRNA UCA1 水平均明显降低, 差异具有统计学意义 (均 $P < 0.05$); 总胆固醇、三酰甘油、空腹血糖差异无统计学意义 (均 $P > 0.05$);

表 5 不同妊娠结局 HDCP 患者生化指标和血清 DNMT1 mRNA, LncRNA UCA1 水平比较 ($\bar{x} \pm s$)

项 目	妊娠结局		t	P
	良好组 ($n=122$)	不良组 ($n=73$)		
白细胞计数 ($\times 10^9/L$)	9.16 ± 2.04	10.03 ± 2.56	2.615	0.010
总胆固醇 (mmol/L)	4.03 ± 1.01	3.95 ± 0.88	0.561	0.575
三酰甘油 (mmol/L)	1.59 ± 0.37	1.61 ± 0.33	0.380	0.704
空腹血糖 (mmol/L)	5.07 ± 0.62	5.18 ± 0.65	1.177	0.240
DNMT1 mRNA	0.80 ± 0.20	0.59 ± 0.15	7.757	< 0.001
LncRNA UCA1	0.72 ± 0.17	0.43 ± 0.14	12.290	< 0.001

2.5 HDCP 患者妊娠结局的影响因素分析 见表 6。以 HDCP 患者是否发生不良妊娠结局为因变量 (未发生 =0, 发生 =1), 将 HDCP 严重程度 (PIH=0, 轻度 PE=1, 重度 PE=2)、收缩压 (实测值)、舒张压 (实测值)、白细胞计数 (实测值)、DNMT1 mRNA (实测值)、LncRNA UCA1 (实测值) 作为自变量建立多因素 Logistic 回归分析, 发现重度 PE、收缩压、舒张压是影响 HDCP 患者发生不良妊娠结局的危险因素, DNMT1 mRNA, LncRNA UCA1 是影响 HDCP 患者发生不良妊娠结局的保护因素 (均 $P < 0.05$)。

表 6 HDCP 患者妊娠结局的影响因素分析

因 素	β	SE	Wald	P	OR	95%CI
重度 PE	0.535	0.183	8.557	0.003	1.708	1.193~2.445
收缩压	0.402	0.161	6.238	0.013	1.495	1.090~2.049
舒张压	0.483	0.209	5.341	0.021	1.621	1.076~2.442
白细胞计数	0.232	0.149	2.439	0.118	1.262	0.942~1.690
DNMT1 mRNA	-0.182	0.071	6.623	0.010	0.833	0.725~0.957
LncRNA UCA1	-0.228	0.068	11.258	0.001	0.796	0.696~0.909

2.6 血清 DNMT1 mRNA, LncRNA UCA1 对 HDCP

患者发生不良妊娠结局的预测价值 见图 1, 表

7. 绘制血清 DNMT1 mRNA, LncRNA UCA1 预测 HDCP 患者发生不良妊娠结局的 ROC 曲线, 发现血清 DNMT1 mRNA, LncRNA UCA1 和二者联合预测 HDCP 患者发生不良妊娠结局的 AUC 分别为 0.832, 0.844 和 0.926, 二者联合预测 HDCP 患者的 AUC 大于 DNMT1 mRNA 及 LncRNA UCA1 单独预测的 AUC ($Z=2.932, 2.345, P=0.003, 0.019$)。

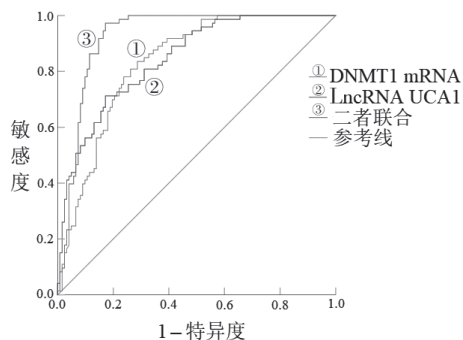


图1 血清 DNMT1 mRNA, LncRNA UCA1 预测 HDCP 患者发生不良妊娠结局的 ROC 曲线

3 讨论

表 7 血清 DNMT1 mRNA, LncRNA UCA1 对 HDCP 患者发生不良妊娠结局的预测价值

指标	AUC	95% CI	截断值	敏感度 (%)	特异度 (%)	约登指数
DNMT1 mRNA	0.832	0.772~0.881	0.74	68.19	82.63	0.508
LncRNA UCA1	0.844	0.785~0.892	0.57	71.23	82.79	0.540
二者联合	0.926	0.879~0.958	-	97.26	81.10	0.784

众所周知, DNA 甲基化异常与多种疾病有关。DNMT1 位于人 19p13.2-3.2, 可维持有丝分裂 DNA 甲基化状态, 并能调节细胞周期^[13]。GENG 等^[14]研究显示, 多囊卵巢综合征患者的颗粒细胞中整体 DNA 甲基化降低, DNMT1 表达下调, 进而抑制细胞周期蛋白依赖性激酶抑制剂 1A 启动子的 DNA 甲基化, 促进多囊卵巢综合征进展。本研究结果显示, 相较于健康妊娠孕妇, HDCP 患者血清 DNMT1 mRNA 水平降低, 表明 DNMT1 mRNA 可能参与 HDCP 发病过程。近期国内学者发现, DNMT1 能抑制 L-NAME 诱导的滋养细胞发生自噬^[5]。相关研究亦证实, 对 L-NAME 诱导的 PE 大鼠模型的胎盘和缺氧处理的滋养层中检测到 DNMT1 表达下调; L-NAME 诱导的滋养层细胞刺激 miR-148/152 并抑制 DNMT1 表达, DNMT1 表达降低与脂肪酸结合蛋白 4 低甲基化有关, miR-148/152 调控 PE 病理过程与 DNA 甲基化有关^[15]。李静等^[16]也发现 DNMT1 水平表达与孕妇不明原因复发性流产有关, 低 DNMT1 水平可能会导致 DNMT1 低甲基化, 进而诱导孕妇流产。本研究中 PIH 者、轻度 PE 者、重度 PE 者血清 DNMT1 mRNA 水平依次降低, 表明 DNMT1 mRNA 水平与 HDCP 病情严重程度相关, 检测 DNMT1 mRNA 水

目前全世界因 HDCP 死亡的孕妇仍居高不下^[8]。HDCP 不仅增加胎盘早剥等不良妊娠结局风险, 还与母体心血管疾病 (心力衰竭、心肌梗死、卒中)、肾脏疾病发生率增加有关, 同时该类群孕妇更易产生抑郁、焦虑情况^[9]。由于妊娠期间宫内环境较差也将影响胎儿正常发育, 增加新生儿精神发育、代谢综合征等疾病易感性^[10]。王莉等^[11]研究指出, HDCP 患者剖宫产、胎膜早破、新生儿窒息、胎儿窘迫、低体重儿、早产比例高于正常妊娠孕妇。相关研究亦发现, HDCP 孕妇伴随较高的早产、胎膜早破、产后出血、胎儿生长受限、胎儿窘迫率^[12]。本研究经统计并比对病例组与对照组不良妊娠结局, 证实与以往研究相符, 表明此类人群伴随有较高不良妊娠结局发生率, 需引起重视。鉴于 HDCP 的危害性极大, 若能实现尽早筛查, 进而有效干预, 有助于预防 HDCP 病情进展, 保证母婴安全。本研究对照组和病例组分娩孕周差异无统计学意义, 可能原因是 HDCP 患者在分娩前进行了相应的治疗, 使得 HDCP 患者分娩孕周与健康妊娠孕妇接近。

平能反映出病情程度, 有助于临床转变治疗策略。推测, DNMT1 mRNA 可能通过调节滋养细胞自噬、以及发病过程中关键因子的甲基化, 参与 HDCP 发生发展。进一步探究 DNMT1 mRNA 与 HDCP 预后的关系, 结果显示, 妊娠结局不良 HDCP 患者血清 DNMT1 mRNA 水平较妊娠结局良好者降低, 提示 DNMT1 mRNA 水平降低与 HDCP 孕妇较差的妊娠结局有关, 其有潜能作为不良妊娠结局预测指标。回归分析显示, DNMT1 mRNA 是影响 HDCP 患者发生不良妊娠结局的保护因素, 推测 DNMT1 mRNA 低表达可能通过调节靶基因, 促进滋养细胞凋亡, 减弱细胞侵袭、增殖能力, 影响胎盘发育, 加重病情, 最终导致不良妊娠结局发生。

LncRNA 已成为滋养细胞和胎盘功能障碍的关键分子调节因子, 通过滋养细胞增殖、凋亡、侵袭等生理活动, 调节血管生成和退膜化、炎症和免疫, 参与 HDCP 发展^[17]。LncRNA UCA1 位于人 19q13.12, 具有原癌基因功能, 最初发现与恶性肿瘤如子宫内膜癌、乳腺癌等癌细胞增殖、耐药、侵袭过程有关^[18]。值得注意的是, 近期发现 LncRNA UCA1 异常表达与滋养细胞分化中基因失调有关, 参与分化、增殖、氧化应激、膜修复等^[19]。本研究发现, HDCP 患者血清 LncRNA UCA1 水

平低于健康妊娠孕妇,且PIH组、轻度PE组、重度PE组LncRNA UCA1水平逐次降低,表明LncRNA UCA1可能参与HDCP发生发展,病情越严重,LncRNA UCA1水平越低。LI等^[20]研究证实,LncRNA UCA1在PIH孕妇中水平降低,这与本研究结果相一致,该研究利用基础实验发现,LncRNA UCA1通过调节PIH中miR-197-3p/组蛋白去乙酰化酶2轴促进人胎盘血管内皮细胞活力和抑制凋亡,增强血管生成能力,进而抑制PIH进展。另有学者发现,LncRNA UCA1在复发性流产患者绒毛组织中下调,LncRNA UCA1过表达增强了滋养细胞和初级滋养层的侵袭、迁移能力^[21]。本研究结果发现,相较于妊娠结局良好组,妊娠结局不良组血清LncRNA UCA1水平降低,且利用回归分析明确了LncRNA UCA1是影响HDCP患者发生不良妊娠结局的保护因素,表明LncRNA UCA1异常水平可能会使妊娠结局受影响,推测低水平LncRNA UCA1可能靶向下游信使RNA或miRNA,影响滋养细胞活性,阻碍胎盘血管新生,加重胎盘内环境恶化,积极推动HDCP进展,最终产生不良妊娠结局。本研究ROC曲线分析显示,血清DNMT1 mRNA,LncRNA UCA1预测不良妊娠结局AUC为0.832,0.844,表明二者均有较佳预测价值;进一步将二者联合,AUC为0.926,敏感度为97.26%,特异度为81.10%,说明联合检测提高了敏感度,降低漏诊率,整体效能提高,有助于临床评估妊娠结局。此外,PAN等^[22]发现,DNMT1介导UCA1启动子DNA低甲基化,激活LncRNA UCA1表达,参与苯或苯二酚致癌过程。本研究Pearson系数显示,HDCP患者血清DNMT1 mRNA,LncRNA UCA1呈正相关,表明DNMT1 mRNA,LncRNA UCA1可能共同调控滋养细胞活性,参与HDCP进展,但具体机制尚不清楚。

综上所述,HDCP患者血清DNMT1 mRNA与LncRNA UCA1水平均降低,二者呈正相关,均与病情程度、妊娠结局相关,DNMT1 mRNA联合LncRNA UCA1检测对不良妊娠结局有较佳预测效能,可辅助临床预测妊娠结局。本研究局限性:未探讨DNMT1 mRNA,LncRNA UCA1之间具体调控机制,Logistic回归模型中纳入分析因素有限,且未动态监测孕前DNMT1 mRNA,LncRNA UCA1水平变化,针对上述问题仍需后期完善研究,并结合基础实验,明确DNMT1 mRNA,LncRNA UCA1参与HDCP具体作用机制,为临床靶向干预提供依据。

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