

妊娠糖尿病患者外周血 miRNA-21 检测的临床应用价值

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摘要:目的 探讨妊娠糖尿病患者外周血 miRNA-21 检测的临床应用价值。方法 选择2016年9月~2019年3月榆林市第三医院妇产科收治的妊娠糖尿病(gestational diabetes mellitus, GDM)孕妇作为GDM组,选择同期在榆林市第三医院进行健康体检的非妊娠糖尿病孕妇为对照组,两组各79例。采集两组受试孕妇外周血,检测空腹血糖、餐后2h血糖值以及miRNA-21表达水平并进行相关性分析。结果 GDM组孕妇的空腹血糖水平、餐后2h血糖水平以及外周血miRNA-21相对表达水平均高于对照组,差异具有统计学意义($t=45.652, 16.663, 148.541$, 均 $P<0.05$); Spearman 相关分析显示外周血中miRNA-21相对表达水平与空腹血糖、餐后2h血糖均呈显著正相关性($r=0.655, 0.732$, 均 $P<0.05$); 二分类Logistic 回归分析显示空腹血糖、餐后2h血糖、外周血miRNA-21均为导致妊娠糖尿病发病的显著影响因素($P<0.05$)。结论 孕妇外周血miRNA-21水平一定程度上可作为其是否患妊娠糖尿病的判断依据。

关键词: 外周血; miRNA-21; 妊娠糖尿病; 血糖

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Clinical Application Value of Detection of miRNA-21 in Peripheral Blood of Patients with Gestational Diabetes

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Abstract: Objective To investigate the clinical application value of detection of miRNA-21 in peripheral blood of patients with gestational diabetes. **Methods** From September 2016 to March 2019, pregnant women with gestational diabetes diagnosed in the Department of Obstetrics and Gynecology of the Third Hospital of Yulin City were selected as the GDM group, and pregnant women with non-pregnancy diabetes who were selected for physical examination in their hospital were selected as the control group, and there were 79 cases in the two groups. Peripheral blood were collected from both groups, and the expression level of miRNA-21 were detected by qRT-PCR and correlation analysis were performed. **Results** The fasting blood glucose, postprandial 2h blood glucose and postoperative miRNA-21 expression levels in the GDM group were higher than those in the control group ($P<0.05$), and the difference was statistically significant ($t=45.652, 16.663, 148.541$, all $P<0.05$). Spearman correlation analysis showed that the relative expression level of miRNA-21 in peripheral blood were significantly positively correlated with fasting blood glucose and postprandial 2h blood glucose ($r=0.655, 0.732$, all $P<0.05$). Logistic regression analysis showed that fasting blood glucose, postprandial 2h blood glucose, and peripheral blood miRNA-21 were the influencing factors of gestational diabetes ($P<0.05$). **Conclusion** The detection level of miRNA-21 in peripheral blood of pregnant women can be used as a basis for judging whether they have GDM to some extent.

Keywords: peripheral blood; miRNA-21; gestational diabetes mellitus; blood glucose

妊娠糖尿病(gestational diabetes mellitus, GDM)是指孕妇在妊娠期首次发生糖耐量受损现象所导致的疾病^[1]。该病严重影响了孕妇的正常妊娠,给孕妇及胎儿均造成了不良影响^[2-3]。微小RNA(microRNA, miRNA)是非编码的一类内源性小分子RNA,广泛地参与细胞分化、凋亡、生

长等过程,其异常表达介导了肿瘤的发生和发展^[4]。相关研究结果表明:miRNA在GDM孕妇和正常孕妇外周血中的表达存在差异^[5],但是具体的作用机制还不明确。鉴于此,本研究以GDM孕妇为研究对象,分析妊娠糖尿病患者外周血中miRNA-21的表达水平与血糖水平的相关性,并探讨了

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miRNA-21 检测的临床应用价值,现总结报道如下。

1 材料与方法

1.1 研究对象 选择2016年9月~2019年3月在榆林市第三医院诊治的GDM孕妇79例作为GDM组,入组患者均符合妊娠糖尿病的诊断标准。同期选择在本院进行健康体检的非妊娠糖尿病孕妇79例作为对照组。纳入标准:孕周8~20周;近3个月均未服用口服避孕药、激素类药物;临床资料完整;年龄20~35岁;单胎妊娠。排除标准:临床资料缺乏者;子宫内膜异位症者;卵巢手术史、盆腔结核史者;存在其他内分泌疾病患者。本研究得到了本院伦理委员会的审批并通过,所有研究对象均知晓本研究内容,并自愿签署了知情同意书。两组孕妇的产次、孕次、孕周、年龄、身高等一般资料对比差异均无统计学意义(均 $P>0.05$)。见表1。

表1 两组一般资料对比($\bar{x} \pm s$)

项目	GDM (n=79)	对照组 (n=79)	t	P
产次(次)	1.55±0.33	1.56±0.21	-0.217	0.829
孕次(次)	2.53±0.18	2.50±0.23	0.872	0.385
孕周(周)	12.40±1.44	12.28±1.98	0.416	0.678
年龄(岁)	26.87±2.44	26.28±3.09	1.272	0.205
身高(cm)	161.33±9.49	162.44±10.00	-0.683	0.493

1.2 试剂和仪器 分别于研究对象清晨空腹状态和餐后2h采集2ml静脉外周血,常规方法进行血糖检测;外周血总RNA的提取采用一步提取法(TRIZOL试剂,北京天根生化科技有限公司),提取的总RNA置于-80℃冻存。使用琼脂糖凝胶电泳检测RNA完整性,选择紫外分光光度计(美国Amersham公司)测定RNA浓度和纯度。

1.3 方法

1.3.1 临床资料调查与血糖指标检测:记录两组孕妇的产次、孕次、孕周、年龄、身高等一般资料,同时检测并记录空腹血糖水平和餐后2h血糖水平。

1.3.2 qRT-PCR 检测 miRNA-21 表达水平:采用实时荧光定量PCR仪(美国Invitrogen公司)行qRT-

PCR。cDNA的合成体系中包含约20~25 ng的总RNA,反应条件:42℃ 1h, 95℃ 5min。PCR反应总体积:20 μl。实时PCR反应条件:95℃预变性10min;然后以95℃ 10s, 60℃ 1min,进行40个循环,扩增反应在实时荧光定量PCR仪上进行。以miRNA-21与内参U6的反应体系中得到的Ct值计算miRNA-21的相对表达水平。miRNA-21上游引物为:5'-CCGCTCGAGGGTAGGAGG-3',下游引物为:5'-GCTAGACCTCTGGGCCTC-3';U6上游引物:5'-GCCAACGTCAGTAGGCAGA-3',下游引物:5'-GCCAACCATGATCTGCTGAAAC-3'。

1.4 统计学分析 采用SPSS22.00对数据进行分析,计量数据以均数±标准差($\bar{x} \pm s$)表示,比较采用卡方检验,以 $P<0.05$ 为差异具有统计学意义。相关性分析采用Spearman相关分析与二分类Logistic回归分析,检验水准为 $\alpha=0.05$ 。

2 结果

2.1 两组血糖水平和 miRNA-21 相对表达水平比较 见表2。与对照组孕妇相比,GDM组孕妇的空腹血糖(FPG)、餐后2h血糖(2hPG)水平以及外周血中miRNA-21相对表达水平均升高,组间比较差异均有统计学意义($P<0.001$)。

表2 两组血糖水平和 miRNA-21 相对表达水平比较($n=79, \bar{x} \pm s$)

项目	GDM	对照组	t	P
FPG (mmol/L)	8.22±0.31	4.87±0.54	45.652	<0.001
2hPG (mmol/L)	16.02±2.84	9.87±1.32	16.663	<0.001
miRNA-21	5.02±0.21	0.87±0.11	148.541	<0.001

2.2 Spearman 相关性分析 Spearman相关性分析结果显示:GDM组孕妇外周血中miRNA-21相对表达水平与空腹血糖、餐后2h血糖水平均呈显著正相关性($r=0.655, 0.732$,均 $P<0.001$)。

2.3 妊娠糖尿病发生的影响因素分析 见表3。空腹血糖、餐后2h血糖水平以及外周血miRNA-21相对表达水平均为导致GDM发病的显著影响因素(均 $P<0.05$)。

表3 妊娠糖尿病发生的影响因素分析

项目	B	SE	Wald	P	OR	95%CI
FPG (mmol/L)	0.204	0.043	22.885	<0.001	0.814	0.333-0.988
2hPG (mmol/L)	0.448	0.032	4.877	0.029	1.478	1.224-9.588
外周血 miRNA-21	0.467	0.205	5.295	0.045	1.672	1.094-5.771

3 讨论

孕妇在妊娠期常因具有拮抗胰岛素功能的激素分泌量增加导致其对胰岛素敏感度降低,因而其在

该时期易患糖尿病,称为妊娠糖尿病(GDM)^[6]。该疾病通常受胰岛素抵抗、炎症因子、胰岛B细胞功能缺陷、遗传、妊娠激素等因素影响,其发生

和进展过程通常伴随着多个具有重要功能的基因表达情况改变,因此表观遗传学机制可能与GDM的发生和发展密切相关^[7-9],GDM的分子调控机制研究对该疾病的早期诊断具有重要意义^[10]。

miRNA 相关研究表明其在基因翻译后的修饰调控中发挥重要作用,可在转录后水平负性调控靶基因的表达,另外,其表达具有高特异度,并可在血液检测作为疾病诊断的标志物^[11-12]。与对照组孕妇相比,GDM组孕妇的空腹血糖、餐后2h血糖水平以及外周血中miRNA-21相对表达水平平均升高,组间比较差异具有统计学意义(均 $P<0.001$),表明GDM孕妇的血糖升高常伴随有外周血miRNA-21相对表达水平升高。本研究中Spearman相关性分析显示GDM孕妇的外周血中miRNA-21相对表达水平与空腹血糖、餐后2h血糖水平均为显著正相关性(均 $P<0.001$);二分类Logistic回归分析显示:GDM孕妇的空腹血糖、餐后2h血糖水平以及外周血miRNA-21相对表达水平均为影响妊娠糖尿病发病的相关因素($P<0.05$)。分析其原因可能在于:miRNA-21在肿瘤的发生发展过程中起到癌基因或抑癌基因的作用^[14],其可直接靶向胰岛素受体、胰岛素受体底物等,从而调控胰岛 β 细胞的生长、存活和有丝分裂,也可促进成熟内皮细胞所介导的血管新生,从而导致机体血糖分泌异常,诱发妊娠糖尿病的发生^[15]。另外,本研究不足之处在于未能进一步研究miRNA-21对妊娠糖尿病影响的具体分子机制,同时样本数量和研究指标较少,因此可能存在一定的研究偏倚性,将在后续研究中进行深入分析。综上所述,GDM患者外周血中miRNA-21呈高表达水平,且其表达水平与血糖水平显著相关,另外,空腹血糖、餐后2h血糖、外周血miRNA-21均为导致妊娠糖尿病发病的影响因素,因此孕妇外周血miRNA-21的检测水平一定程度上可作为其是否患妊娠糖尿病的判断依据。

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