

doi: 10.13241/j.cnki.pmb.2019.22.031

外周血 miR-223 和血清 BNP 水平在慢性心力衰竭患者中的诊断价值*

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摘要 目的:探究慢性心力衰竭(chronic heart failure, CHF)患者外周血 microRNA-223(miR-223)和血清 B 型利钠肽(B-type natriuretic peptide, BNP)水平及其诊断价值。**方法:**选取 CHF 患者 65 例(CHF 组),其中美国纽约心脏病协会(New York Heart Association, NYHA)心功能分级 II 级 24 例, III 级 22 例, IV 级 19 例。另取 40 例同期在体检中心进行健康体检者(Control 组),采用实时荧光定量 PCR 检测外周血中 miR-223 水平, ELISA 检测血清 BNP 含量, ROC 曲线评价 miR-223 及 BNP 对 CHF 的诊断价值。**结果:**CHF 组左心室短轴缩短率及左心室射血分数显著低于 Control 组($P<0.01$);左心室舒张末期径显著高于 Control 组($P<0.01$)。CHF 组不同 NYHA 心功能分级患者 miR-223 和 BNP 水平均高于 Control 组,且 miR-223 和 BNP 水平随 NYHA 心功能分级逐渐递增,组间两两比较均显示统计学差异($P<0.05$)。miR-223 诊断 CHF 的曲线下面积(area under the curve, AUC)为 0.7375, 临界值为 43.51 时灵敏度为 92.82%, 特异度为 89.44%;BNP 诊断 CHF 的 AUC 为 0.7925, 临界值为 128 ng/L 时灵敏度为 92.93%, 特异度为 92.58%。**结论:**CHF 患者外周血 miR-223 和血清 BNP 水平高于健康对照人群,其对 CHF 的诊断具有一定的临床参考价值。

关键词:心力衰竭;miR-223;B 型利钠肽;诊断**中图分类号:**R541.61; R540.4 **文献标识码:**A **文章编号:**1673-6273(2019)22-4344-04

Diagnostic Value of Peripheral Blood miR-223 and Serum BNP Levels for the Patients with Chronic Heart Failure*

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ABSTRACT Objective: To investigate the level of microRNA-223 (miR-223) in peripheral blood and serum B-type natriuretic peptide (BNP) level of patients with chronic heart failure (CHF), and to analyze their diagnostic value. **Methods:** Sixty-five patients with CHF were enrolled in the CHF group: 24 patients with New York Heart Association (NYHA) functional class II, 22 with class III and 19 with class IV. Another 40 healthy subjects who underwent a physical examination were included in the control group. The level of miR-223 in peripheral blood was measured by real-time quantitative PCR. The serum BNP was determined by ELISA. The ROC was used to evaluate the diagnostic value of miR-223 and BNP for CHF. **Results:** Compared to the control group, the left ventricular ejection fraction and fractional shortening were significantly lower, left ventricular end-diastolic diameter was significantly wider in the CHF group (all $P<0.01$). The level of miR-223 or BNP in the patients with CHF was higher than that in the control group ($P<0.05$), and the level of miR-223 or BNP gradually increased with NYHA functional class. The area under the curve (AUC) of miR-223 to diagnose CHF was 0.7375; while the cutoff of miR-223 was 43.51, sensitivity and specificity were 92.82% and 89.44%. The AUC of BNP was 0.7925; while the cutoff of BNP was 128 ng/L sensitivity and specificity were 92.93% and 92.58%. **Conclusions:** The level of miR-223 in peripheral blood and serum BNP level of patients with CHF are up-regulated, which had a good diagnostic value for CHF.

Key words: Heart failure; miR-223; B-type natriuretic peptide; Diagnosis**Chinese Library Classification(CLC):** R541.61; R540.4 **Document code:** A**Article ID:** 1673-6273(2019)22-4344-04

* 基金项目:国家自然科学基金面上项目(81670365)

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(收稿日期:2019-08-08 接受日期:2019-08-31)

前言

microRNAs(miRNAs)是一组长度约为22个核苷酸,高度保守的非编码的单链小RNA分子,通过与靶mRNA结合调控基因表达。由于循环miRNA能够抵抗核糖核酸酶降解,在反复冻融及极端pH条件下稳定存在,miRNA的检测被广泛应用于恶性肿瘤的诊治^[1,2]。近年来,miRNA被证实参与多种心血管疾病的发生和发展,已成为潜在的心血管疾病诊断标记物和新的治疗靶点^[3-6]。miR-223在心血管系统的作用已被报道,Gou等研究发现miR-223与小鼠病毒性心肌炎密切相关^[7];Yu等研究发现miR-223与心肌缺血再灌注损伤有关^[8,9];亦有学者发现miR-223转基因鼠可发展为心肌肥厚和心力衰竭^[10]。但有关慢性心力衰竭(chronic heart failure, CHF)患者miR-223的表达情况目前鲜有报道。本研究通过对比分析健康人群和CHF患者外周血中miR-223和B型利钠肽(B-type natriuretic peptide, BNP)的表达水平,探讨其在CHF诊断中的临床价值。

1 对象与方法

1.1 研究对象

选取2018年1月至2019年6月在西安医学院第二附属医院心内科住院治疗的CHF患者65例(CHF组)。CHF判定采用2016年欧洲心脏病学会发布的心力衰竭诊治指南^[11]。所有患者排除血液系统疾病、肝肾功能异常、严重内分泌性疾病、免疫性疾病、感染性疾病、肺动脉高压、急性心肌梗死、恶性肿瘤者。另选取同期在西安医学院体检中心进行健康体检者40例作为Control组。本研究所有入选者均签署知情同意书。

1.2 超声心动图

采用Philips Sonos7500超声诊断仪,连续3次记录心动周期,取平均值。胸骨旁左心室长轴切面测量左心室舒张末内径(left ventricular end-diastolic diameter, LVEDD),左心室收缩末内径(left ventricular end-systolic diameter, LVESD),左心室后壁厚度(left ventricular posterior wall thickness, LVPWT)并计算左心室短轴缩短率(fractional shortening, FS)=(LVEDD-LVESD)/LVEDD×100%。心尖四腔和两腔切面测量左心室收缩末期容积和舒张末期容积,双平面Simpson法计算左心室射血分数(left ventricular ejection fraction, LVEF)=(左心室舒张末期容积-左心室收缩末期容积)/左心室舒张末期容积×100%^[12]。

1.3 血清BNP水平检测

清晨空腹状态下用无抗凝剂真空管采集静脉血,室温静置3h后,4℃离心机3000rpm离心20min,抽取上清,即分得血清,分装后置于-80℃超低温冰箱中保存。采用酶联免疫试验法(ELISA)检测血清中BNP含量,试剂盒购买于美国Abcam公司,仪器采用Bio-Tek全波长酶标仪进行检测,参照试剂盒说明进行操作。

1.4 RNA的提取和实时荧光定量PCR(real time quantitative-PCR, RT-qPCR)

总RNA提取按照总RNA提取试剂盒(北京索莱宝科技有限公司)说明书要求进行操作,反转录按照Mir-X miRNA RT-qPCR TB Green™Kit(Takara 日本)反转录试剂盒的说明书

进行。引物合成由上海生工完成,U6引物序列:正向引物5'-ATTGGAACGATACAGAGAAGATT-3',反向引物5'-GGAACGCTTCACGAATTTG-3;miR-223引物序列:正向引物5'-ACACTCCAGCTGGGTGTCAGTTTGTCAAAT-3',反向引物5'-TGGTGTCTGGATTCTG-3'。反应条件分别如下:16℃,30min;42℃,30min;75℃15min,将产物cDNA置于-20℃供长期使用。用U6作为内部参考基因,扩增条件为:94℃,3min;94℃,35s;60℃,35s;72℃,60s;共40个循环。

1.5 统计学分析

采用SPSS17.0统计软件进行统计分析。计量资料采用Kolmogorov-Smirnov正态性检验,正态分布的计量资料以平均值±标准差($\bar{x} \pm s$)表示,非正态分布的计量资料对数据进行自然对数转换转为正态分布资料,两组比较采用t检验,多组比较采用方差分析;计数资料表示为计数和百分比(n,%),根据样本量大小采用 χ^2 检验或Fisher精确检验。采用受试者工作特征曲线(Receiver operating characteristic curve, ROC曲线)与曲线下面积(Area under curve, AUC)评估诊断价值。以 $P < 0.05$ 为具有统计学差异。

2 结果

2.1 两组患者一般临床资料的比较

CHF组男性36例,女性29例,年龄55~78岁;Control组男性22例,女性18例,年龄53~72岁,两组间性别、年龄、体重指数、糖尿病史、吸烟、饮酒情况和LVPWT一般临床资料比较均无统计学差异($P > 0.05$);而CHF组FS及LVEF显著低于对照组($P < 0.01$);LVEDD显著高于对照组($P < 0.01$),见表1。

2.2 两组外周血中miR-223和血清BNP水平的比较

将CHF组患者按照美国纽约心脏病协会(New York Heart Association, NYHA)心功能分级分为NYHA心功能II级24例,III级22例,IV级19例。CHF组不同心功能分级患者外周血miR-223(图1)和BNP(图2)水平均高于对照组,且miR-223和BNP水平随NYHA心功能分级逐级递增,组间两两比较均显示统计学差异($P < 0.05$)。

2.3 ROC曲线分析外周血miR-223和血清BNP水平对CHF的诊断价值

ROC曲线分析外周血miR-223和血清BNP水平对CHF的诊断价值,结果显示miR-223的AUC为0.7375, $P < 0.01$,临界值为43.51时敏感性为92.82%,特异性为89.44%;BNP的AUC为0.7925, $P < 0.01$,临界值为128ng/L时敏感性为92.93%,特异性为92.58%(图3)。

3 讨论

CHF是各种心脏疾病的严重表现或终末期阶段,具有较高的病死率和再住院率,已经成为严重的公共社会问题^[13]。最新的指南指出生物标记物如BNP或NT-proBNP的提前筛查和干预可以预防或延缓CHF的发生和发展,也有助于对疗效的观察和判断^[14]。然而,由于血液中蛋白成分复杂,蛋白翻译后修饰多样,许多蛋白本身含量较低,以及发展高敏感性检测技术仍具有一定的困难^[15]。越来越多的研究表明在癌症和心血管疾

表 1 CHF 组和 Control 组的一般临床资料比较 $[\bar{x} \pm s, n (\%)]$ Table 1 Comparison of the clinical characteristics between CHF group and Control group $[\bar{x} \pm s, n (\%)]$

	CHF group (n=65)	Control group (n=40)	t/χ^2	P
Male/Female	36/29	22/18	0.12	0.31
Age (years)	66.22 $\pm$ 11.31	63.98 $\pm$ 9.64	1.12	0.30
BMI (kg/m ²)	23.42 $\pm$ 0.54	23.53 $\pm$ 0.62	0.92	0.30
Diabetes mellitus (n, %)	9 (13.85)	4 (10.00)	0.07	0.78
Smoking history (n, %)	3 (4.61)	4 (10.00)	0.06	0.62
Alcohol consumption history (n, %)	4 (6.15)	5 (12.5)	10.52	0.80
FS (%)	21.24 $\pm$ 3.35	32.59 $\pm$ 6.11	6.09	<0.01
LVEF (%)	42.44 $\pm$ 15.22	63.67 $\pm$ 10.43	5.12	<0.01
LVEDD (mm)	64.00 $\pm$ 5.97	57.11 $\pm$ 1.07	8.22	<0.01
LVPWT (mm)	11.47 $\pm$ 1.79	11.15 $\pm$ 1.38	0.94	0.07

Note: BMI: body mass index; CHF, chronic heart failure; FS, fractional shortening; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVPWT, left ventricular posterior wall thickness.

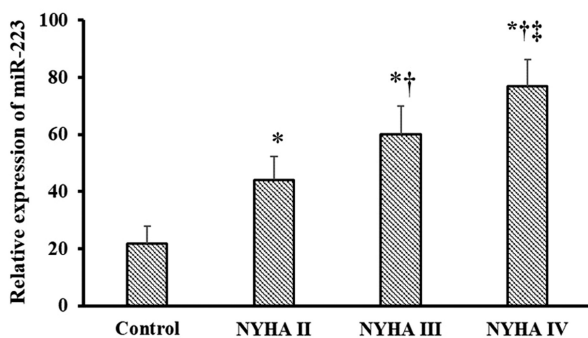


图 1 不同 NYHA 心功能分级患者和 Control 组外周血 miR-223 水平
Fig.1 Relative expression of miR-223 in the peripheral blood of patients with different NYHA functional class and control group

Note: NYHA, New York Heart Association; *:compared with Control group, $P<0.05$; †:compared with NYHA functional class II, $P<0.05$; ‡: compared with NYHA functional class III, $P<0.05$.

病中,miRNA 作为诊断和预后的生物标志物发挥着重要作用^[1-6]。Mitchell 等报道在外周血中可以检测到 miRNA,并有可能成为前列腺癌患者的生物标记物,同时证明在室温下长时间放置和 / 或多次冷冻 - 解冻后, 血浆 miRNA 仍具有很高的稳定性^[16]。miRNA 除了这种高稳定性外,与其他核酸(如循环 DNA 和信使 RNA)相比每个细胞还具有多拷贝性,这些特征使其具备成为生物标记物的潜在优势。

既往的研究证实不同的疾病或细胞中 miR-223 的表达并不一致:肺癌细胞和急性胰腺炎的腹腔透析液中 miR-223 呈高表达^[17,18],类风湿性关节炎患者 T 淋巴细胞中 miR-223 呈过度表达^[19],而急性髓性白血病和痴呆患者 miR-223 的表达受抑制^[20-22]。在心血管系统疾病中,心脏结节病患者 miR-223 表达增加,可能与心脏结节病患者 T 细胞过度刺激有关^[23]。急性心肌梗死患者 miR-223 表达水平明显升高^[24],近期的研究证实 miR-223 是心梗后心肌缺血再灌注损伤的预测因子^[8,9]。Guo 等报道 miR-233 与小鼠病毒性心肌炎密切相关^[7]。这些研究均证实 miR-223 与心血管疾病的发生发展密切相关。既往的研究显

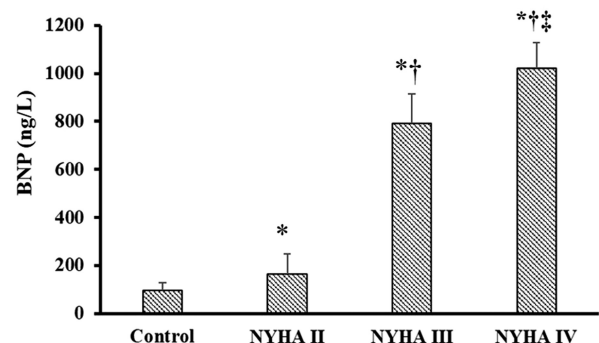


图 2 不同 NYHA 心功能分级患者和 Control 组血清 BNP 水平
Fig. 2 Serum BNP level in patients with different NYHA functional class and control group

Note: BNP, B-type natriuretic peptide; NYHA, New York Heart Association; *: compared with Control group, $P<0.05$; †: compared with NYHA functional class II, $P<0.05$; ‡: compared with NYHA functional class III, $P<0.05$.

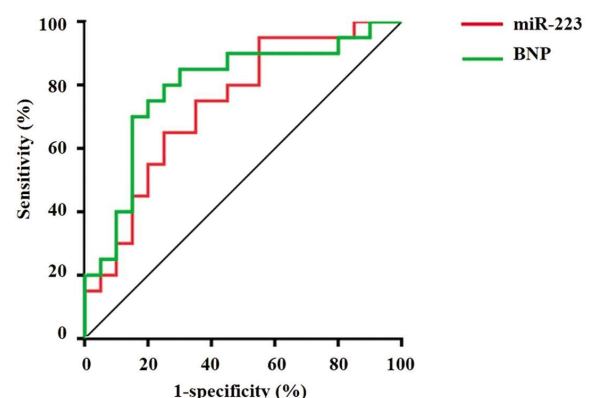


图 3 ROC 曲线分析外周血 miR-223 和血清 BNP 水平对 CHF 的诊断价值

Fig. 3 ROC of peripheral blood miR-223 and serum BNP level for the diagnosis of CHF

Note: BNP, B-type natriuretic peptide; CHF, chronic heart failure; ROC, receiver operating characteristic.

示多种 miRNA 与 CHF 相关,2011 年 Kumarswamy 等^[25]报道 CHF 患者 miR-21 表达明显高于正常人。此后,先后有研究证实 miR-126、miR-150、miR-210、miR-425 及 miR-744 等多种 miRNA 表达与 CHF 的发生发展有关^[4,26-30]。虽然有动物研究发现 miR-223 转基因鼠可发展为心肌肥厚和心力衰竭^[10],但临床 CHF 患者 miR-223 的表达情况目前仍未有报道。

本研究通过检测 CHF 患者外周血 miR-223 和血清 BNP 的表达水平,发现随着 NYHA 心功能分级的递增,CHF 患者外周血 BNP 和血清 miR-223 的水平逐级增加,这一发现提示 miR-223 和 BNP 与心力衰竭密切相关。ROC 曲线分析显示 miR-223 和 BNP 均具有 CHF 的诊断价值 (P 均 <0.01),但与 BNP 相比,外周血 miRNA 不仅具有半衰期长、稳定性高的特性,而且其通过组织和病理特异性的方式被调节,能够被序列特异性的扩增方式如 RT-PCR 和基因芯片快速、精确检测,因此 miR-223 对 CHF 的诊断具有一定的优越性^[15]。

总之,CHF 患者外周血 miR-223 水平高于健康对照人群,其对 CHF 的诊断具有一定的临床参考价值,有望成为诊断 CHF 和判断患者预后的重要生物标志物。由于本次研究住院患者结果可能缺乏广泛的代表性,需要进一步多中心大样本的研究支持。

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