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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（$P<0.01$）；左心室舒张末期内径显著高于 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

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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)=(左心室舒张末容积 - 左心室收缩末容积)/ 左心室舒张末容积× 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 3 h 后,4℃离心机 3000 rpm 离心 20 min,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,参照试剂盒说明书进行操作。

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'-TGGTGTCGTGGATTCG-3'。反应条件分别如下:16℃,30 min;42℃,30 min;75℃ 15 min,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℃,3 min;94℃,35 s;60℃,35 s;72℃,60 s;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 x^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 128 ng/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/x^2	*P*
Male/Female	36/29	22/18	0.12	0.31
Age (years)	66.22± 11.31	63.98± 9.64	1.12	0.30
BMI (kg/m^2)	23.42± 0.54	23.53± 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± 3.35	32.59± 6.11	6.09	＜0.01
LVEF (%)	42.44± 15.22	63.67± 10.43	5.12	＜0.01
LVEDD (mm)	64.00± 5.97	57.11± 1.07	8.22	＜0.01
LVPWT (mm)	11.47± 1.79	11.15± 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.

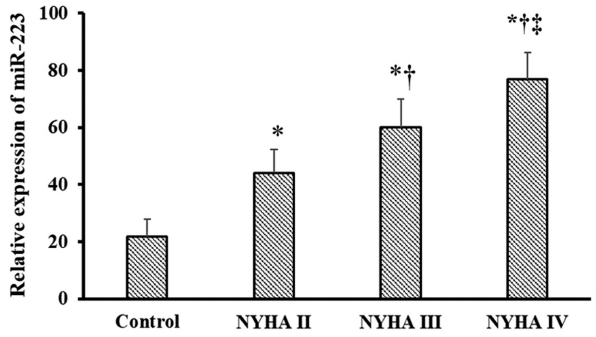

图 1 不同 NYHA 心功能分级患者和 Control 组外周血 miR-223 水平

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.

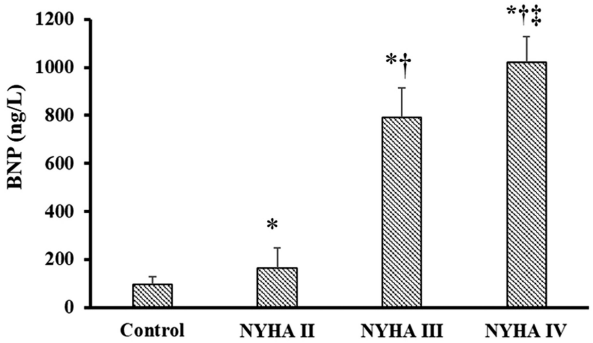

图 2 不同 NYHA 心功能分级患者和 Control 组血清 BNP 水平

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.

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：肺癌细胞和急性腹膜炎的腹腔透析液中 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

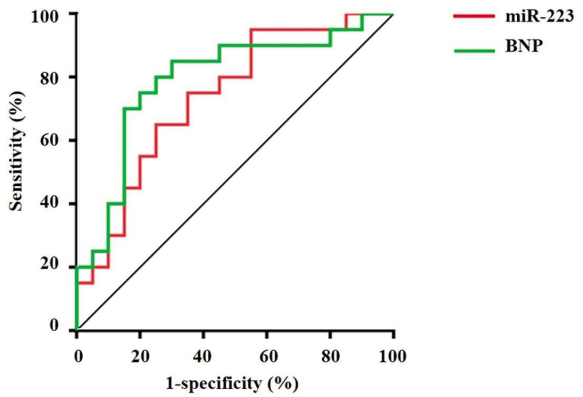

图 3 ROC 曲线分析外周血 miR-223 和血清 BNP 水平对 CHF 的诊断价值

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。

本次研究通过检测 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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