内皮素-1和结缔组织生长因子在心房颤动患者中高表达并与射频消融术后的复发相关

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摘要:目的 探讨内皮素-1(ET-1)和结缔组织生长因子(CTGF)在心房颤动(AF)中的表达变化及对射频消融术后复发的预测价值。**方法** 选取蚌埠医学院第一附属医院心内科就诊的阵发性房颤(PaAF)患者66例和持续性房颤(PeAF)患者72例为实验组,80例窦性心律者为对照组,采用ELISA和Western blot分别检测血清中ET-1和CTGF的表达水平;同时选取我院心脏外科行手术的房颤和窦性心律患者各6例,术中取右心耳组织,采用HE染色和Masson染色分别观察心肌细胞结构形态和心肌纤维化程度,免疫组织化学染色和Western blot检测ET-1和CTGF蛋白表达情况;对实验组行射频消融术的患者开展了为期6个月的临床随访,以评估预后情况。**结果** 与对照组(CON)相比,AF组心肌细胞结构明显被破坏、纤维化程度显著增加;PaAF和PeAF组ET-1和CTGF表达水平明显高于CON组,且PeAF组明显高于PaAF组。在所有实验组中ET-1和CTGF水平与LAD大小呈正相关(P<0.05),ET-1与CTGF水平呈显著正相关(P<0.05)。同时,房颤复发组(Re)患者术后ET-1、CTGF水平均高于未复发组(NRe),术前、术后的ET-1和CTGF表达水平与PeAF术后复发呈正相关,且ET-1、CTGF为PeAF术后复发的独立危险因素。**结论** ET-1和CTGF在AF患者中表达明显增加,与AF持续时间呈正相关;ET-1和CTGF在AF复发组患者中表达水平明显升高,且二者对PeAF患者射频消融术后复发有预测价值。

关键词:内皮素-1;结缔组织生长因子;心房颤动;临床预后;射频消融

Serum levels of endothelin-1 and connective tissue growth factor are elevated in patients with atrial fibrillation and correlated with relapse following radiofrequency ablation

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Abstract: Objective To investigate the changes in serum levels of endothelin-1 (ET-1) and connective tissue growth factor (CTGF) in patients with atrial fibrillation (AF) and their value for predicting recurrence of AF after radiofrequency ablation (RFCA). **Methods** Sixty-six patients with paroxysmal AF (PaAF) and 72 with persistent AF (PaAF) admitted in our hospital were recruited as AF group and 80 patients with sinus rhythm as the control group, and in all the participants, serum levels of ET-1 and CTGF were measured using ELISA and Western blotting. From 6 patients with AF and 6 with sinus rhythm undergoing cardiac surgery in our hospital, tissue samples of the right atrial appendage were taken intraoperatively for observation of structural changes of the cardiomyocytes, myocardial fibrosis and expression of ET-1 and CTGF protein. In AF group, the patients receiving RFCA were followed up for 6 months following the procedure for assessment of the outcomes. **Results** Compared with the control patients, the patients with AF showed obvious damages of the cardiomyocyte structure and myocardial fibrosis. Serum levels of ET-1 and CTGF levels were significantly higher in PaAF and PeAF groups than in the control group, and were higher in PeAF group than in PaAF group. In the patients with AF, serum ET-1 and CTGF levels were positively correlated with left atrial diameter (LAD) (P<0.05), and ET-1 was positively correlated with CTGF levels (P<0.05). In patients with postoperative AF recurrence, the serum levels of ET-1 and CTGF were significantly higher than those in patients without recurrence; serum ET-1 and CTGF levels before and after the operation were positively correlated with the recurrence of PeAF, and elevated serum levels of ET-1 and CTGF were identified by logistic regression analysis as independent risk factors for postoperative recurrence of PeAF. **Conclusion** Serum levels of ET-1 and CTGF are significantly elevated in AF patients in positive correlation with AF duration. ET-1 and CTGF levels are higher in AF patients with postoperative recurrence, and they both have predictive value for recurrence of PeAF following RFCA.

Keywords: endothelin-1; connective tissue growth factor; atrial fibrillation; clinical prognosis; radiofrequency ablation

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心房颤动(AF)是心血管疾病中较为常见的一种,现阶段对AF的防治仍是一大世界性难题[1-3]。AF不仅与心力衰竭和心肌梗死等疾病之间相互影响,还是脑血管疾病的重要危险因素[4-6]。因此,加强对AF的认识越来越受到研究者的重视。现阶段AF的发病机制尚不完全明确,心肌纤维化目前被认为是AF发生、发展的最重要因素之一[7-9],以此为切入点可能成为AF防治的一个

新的方向。

内皮素-1(ET-1)具有强大的缩血管作用和促纤维化作用,在多种组织、器官纤维化的发生机制中发挥关键作用,特别是促进心肌纤维化方面[10, 11]。结缔组织生长因子(CTGF)做为一种多功能生物因子,具有特异性的促进成纤维细胞分裂和胶原沉积作用,可引起组织器官纤维化[12, 13]。Chen等[14]研究证实在小鼠肺纤维化模型中,ET-1可诱导CTGF的表达,进一步促进肺组织纤维化。

ET-1和CTGF二者关系密切,目前认为ET-1和CTGF在高血压病、肺动脉高压、冠心病以及心力衰竭等多种心血管疾病中发挥重要作用,但关于ET-1和CTGF在AF中的作用尚不完全明确,且ET-1和CTGF是否通过诱导心肌纤维化影响AF的发生、发展尚未见报道。本研究拟探讨ET-1和CTGF与AF不同类型的相关性及其对AF患者射频消融术后复发的预测价值,旨在为AF的诊断和防治提供新的思路。

1 资料和方法

1.1 研究对象

选取2020年7月~2022年1月于蚌埠医学院第一附属医院心内科住院治疗的138例心房颤动患者,其中阵发性房颤66例,持续性房颤72例。同时选取同期体检的窦性心律者80例纳入对照组。纳入标准:所有患者经体表心电图记录到房颤心电图可诊断为房颤。阵发性房颤:在发病7 d内自动终止或干预终止的房颤;持续性房颤:持续时间超过7 d的房颤,包括>7 d后通过复律(药物或电复律)终止发作;房颤的分型诊断以2019欧洲心脏病学会(ESC)提出的分型诊断标准为基础依据[15]。排除标准:排除合并风湿性心脏病、左心耳封堵后或左心耳手术结扎/切除术后患者、心脏占位性病变(如心房黏液瘤)、感染性疾病、自身免疫疾病、急性冠状动脉综合征、脑卒中、恶性肿瘤、甲状腺功能异常、左室射血分数<40%、肝肾功能损伤等严重原发性疾病及近期服用过任何抗炎药物的患者。所有手术患者均由本科室两名熟练的电生理组专业医师行射频消融术,射频消融的经线一致、放电参数一致,术后均规范化服用胺碘酮、利伐沙班治疗。另选择2020年8月~2021年2月于我院心脏外科住院的12例心脏外科手术患者为研究对象,按照上述纳入和排除标准,分为房颤组6例和对照组6例,在术中提取右心耳组织行HE染色、Masson染色、免疫组织化学染色及Western blot。所有入组者均自愿签署知情同意书,并获得蚌埠医学院第一附属医院伦理委员会批准(2019KY023)。

1.2 外周血标本的获取和单个核细胞分离

66例阵发性房颤患者、72例持续性房颤患者以及80例同期体检的窦性心律患者均于清晨空腹采集肘静脉血约5 mL,静置15 min后3000 r/min离心15 min,收集血清置于−80 ℃冰箱中保存。在离心管加入淋巴细胞分离液(稀释血:淋巴细胞分离液=1:1);按照试剂说明书严格操作,分离出单个核细胞,加入配置好的血清(胎牛血清:二甲基亚砜=9:1)重悬细胞,转移至冻存管,−80 ℃冰箱保存。

1.3 ELISA法检测对照组、阵发性房颤组、持续性房颤组患者血清中ET-1和CTGF水平

Human ET-1、CTGF检测试剂盒由上海羽朵公司提供,检测方法根据试剂盒中的操作步骤实施,测定人血清ET-1、CTGF表达水平。

1.4 HE染色、Masson染色和免疫组织化学染色

选取我院心脏外科行手术的6例房颤患者和6例窦性心律患者,术中提取100 mg右心耳组织,部分放入液氮中冻存,部分用4%多聚甲醛固定。HE染色法评价右心耳组织的形态学变化。Masson染色法评价心房纤维化的程度。免疫组织化学染色评估ET-1(武汉塞维尔生物科技有限公司)及CTGF(武汉塞维尔生物科技有限公司)在右心耳组织中的表达。将右心耳组织固定和包埋后,切取厚度为5 μm的薄片,严格按照试剂盒说明书进行HE染色、Masson染色和免疫组织化学染色。切片在光学显微镜下进行观察、拍照。纤维化组织被染成蓝色,计算胶原蛋白体积分数(CVF,%)。

1.5 Western blot检测ET-1和CTGF蛋白在对照组、阵发性房颤组、持续性房颤组患者中的表达情况

将已分装好的单个核细胞和右心耳组织分别提取总蛋白,十二烷基硫酸钠聚丙烯酰胺凝胶电泳(SDS-PAGE),湿法转膜,抗体稀释比:抗ET-1鼠单克隆抗体(Abcam)1∶500、抗CTGF兔单克隆抗体(Abcam)1∶1000、抗β-actin兔单克隆抗体(Abcam)1∶10 000,孵育一抗(4 ℃过夜),封闭2 h,TBST洗涤3次,孵育二抗(室温1~2 h),TBST洗涤3次,显影,凝胶成像系统获取图像。

1.6 临床随访

对选择行射频消融术的AF患者进行为期6月的复发情随访,房颤复发定义为随访期内心电图和/或24 h动态心电图证实房颤发作持续时间≥30 s,通过住院或门诊途径,对比患者射频消融术前、术后6月血清中ET-1和CTGF表达情况,评估ET-1和CTGF水平与AF患者射频消融术后复发率之间的关系。

1.7 统计学分析

采用SPSS 26.0软件进行数据处理,计量资料以均数±标准差表示,计数资料以n(%)表示,组间比较行χ^2检验;组间比较采用t检验、方差分析、非参数检验或Fisher确切概率法,单因素相关分析采用Pearson相关分析,以P<0.05为差异有统计学意义。ImageJ 8.0软件分析Western blot条带灰度值及Masson染色胶原蛋白

体积分数，Graphpad-prism 9.0进行统计学分析。二元logistic回归分析AF发病的危险因素，$P<0.05$认为差异具有统计学意义。所有的实验都是独立重复3次。

2 结果

2.1 一般资料比较

各组基线资料显示：患者患者年龄、性别、是否合并高血压病、是否合并糖尿病、是否合并脑梗塞、是否服用抗凝药物、是否服用胺碘酮、红细胞、白细胞、血小板、BNP、LVEF、ALT、AST、血肌酐及低密度脂蛋白差异均无统计学意义（$P>0.05$，表1）。

2.2 HE染色、Masson染色和免疫组织化学染色

表1 对照组、阵发性房颤组、持续性房颤组患者基线资料比较

Tab.1 Baseline data of the patients in the control group, paroxysmal AF (PaAF) group and persistent AF (PeAF) group

Variable	CON (*n*=80)	PaAF (*n*=66)	PeAF (*n*=72)	F/χ^2	*P*
Age (year)	63.85±10.29	65.02±11.12	66.71±8.95	1.52	
Male (*n*, %)	44 (55)	36 (54.55)	43 (65.15)	0.48	
Smoking (*n*, %)	16 (20)	11 (16.67)	15 (20.83)	0.43	
Hypertension (*n*, %)	42 (52.5)	29 (43.94)	40 (55.56)	1.99	
Diabetes mellitus (*n*, %)	6 (7.5)	8 (12.12)	12 (16.67)	3.03	
Cerebral infarction (*n*, %)	15 (18.75)	10 (15.15)	16 (22.22)	1.13	
Anticoagulant (*n*, %)	0 (0)	66 (100)	72 (100)	-	
Amiodarone (*n*, %)	0 (0)	66 (100)	72 (100)	-	
RBC/($\times10^{12}$/L)	4.47±0.36	4.40±0.64	4.36±0.8	0.59	>0.05
WBC/($\times10^{9}$/L)	5.80±1.40	5.85±1.38	6.32±1.91	2.39	
PLT/($\times10^{9}$/L)	204.63±53.82	216.18±56.18	200.79±49.38	1.56	
BNP (pg/mL)	51.57±39.98	53.11±32.41	45.91±21.09	1.11	
LVEF (%)	56.56±5.05	54.85±6.62	56.95±5.91	2.64	
ALT (U/L)	23.26±5.86	22.05±11.69	22.42±9.87	1.18	
AST (U/L)	23.79±7.64	26.98±13.59	26.79±11.98	1.95	
SCR (μmol/L)	63.94±8.67	63.64±14.80	66.71±16.85	1.10	
LDL (mmol/L)	2.50±0.52	2.33±0.66	2.49±0.86	1.32	

RBC: Red blood bell, WBC: White blood bell, PLT: Platelet, BNP: Brain natriuretic peptide, LVEF: Left ventricular ejection fraction, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, SCR: Serum creatinine, LDL: Low-density lipoprotein.

与CON组相比，HE染色结果显示，AF组心肌纤维紊乱，结构破坏明显；Masson染色结果显示，AF组患者右心耳胶原纤维沉积显著（图1A），并计算胶原蛋白体积分数（CVF，%）（图1B）。免疫组织化学染色及IOD值相对定量分析结果显示AF组ET-1和CTGF表达显著高于CON组（图1C、D）。

2.3 血清ET-1、CTGF表达水平和LAD大小比较

ELISA结果表明，同CON组相比，AF组患者血清ET-1、CTGF表达水平和左房内径（LAD）大小增加，差异有统计学意义（$P<0.01$，表2）；PaAF患者ET-1、CTGF表达水平和LAD大小较PeAF患者升高，差异有统计学意义（$P<0.01$，表3）。

2.4 各组血清单个核细胞中ET-1和CTGF蛋白表达情况

Western blot的结果表明，与CON组相比，PaAF组和PeAF组血清单个核细胞中ET-1、CTGF蛋白表达增加；与PaAF组相比，PeAF组ET-1、CTGF蛋白表达增加（图2）。

2.5 房颤组及对照组右心耳组织中ET-1和CTGF蛋白的表达情况

Western blot的结果显示，与CON组相比，AF组血清中ET-1和CTGF蛋白表达增加（$P<0.01$，图3）。

2.6 ET-1、CTGF表达水平与LAD的相关性分析及散点图

Pearson相关性分析及散点图结果显示，AF患者血清ET-1、CTGF表达水平与LAD呈正相关（rET-1=0.18，rCTGF=0.39，$P<0.05$，图4A），ET-1和CTGF水平呈正相关（$r=0.47$，$P<0.05$，图4B）。

2.7 二元logistic回归分析

纳入ET-1、CTGF和LAD行logistic回归分析（均以实测值赋值），结果显示，ET-1、CTGF和LAD均为影

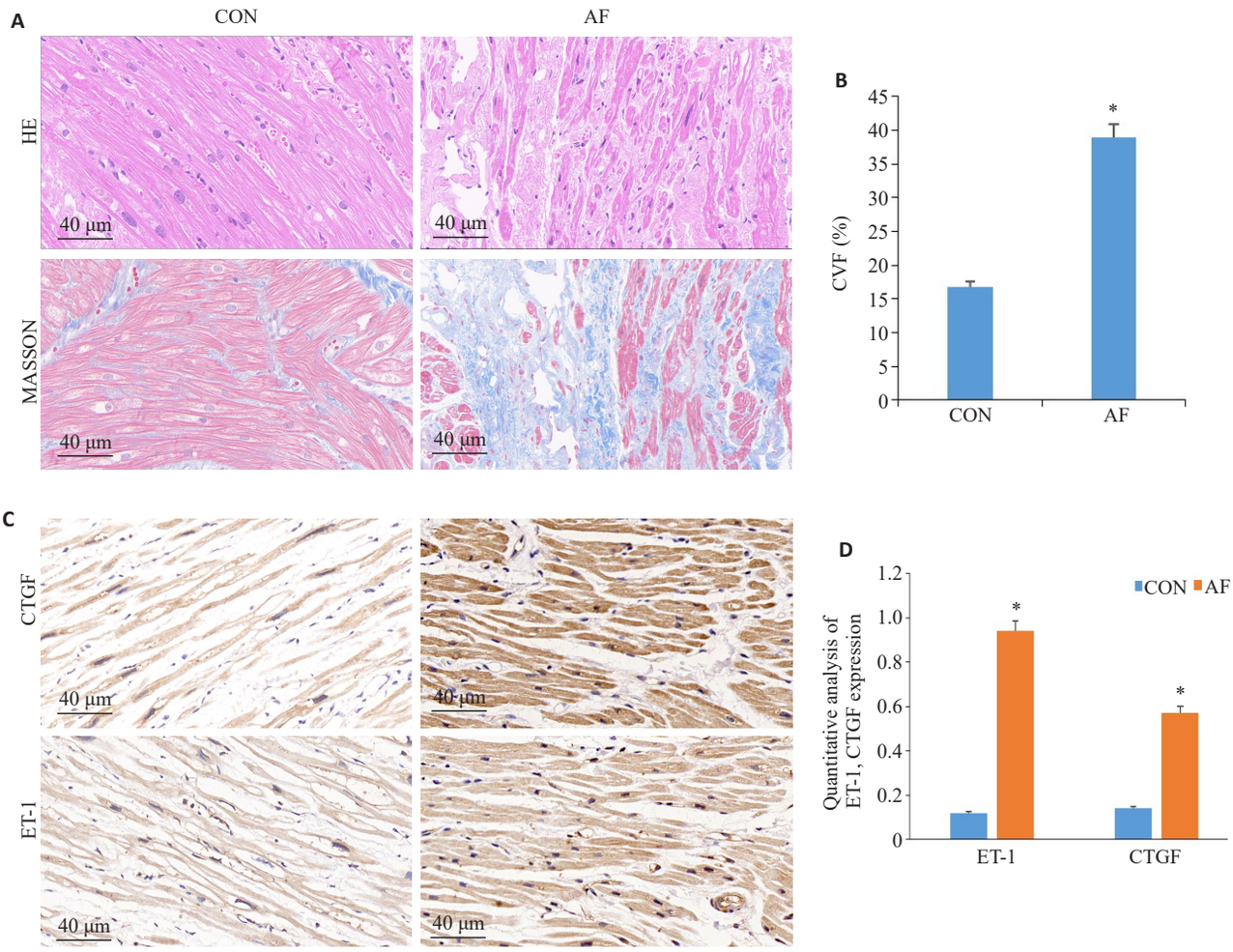

图1　各组心肌细胞HE染色、Masson染色和免疫组织化学染色的结果

Fig.1　Results of HE staining, Masson staining and immunohistochemical staining of myocardial tissues in each group. **A**: HE staining and Masson staining of the myocardial tissues in control and AF groups. **B**: Collagen volume fraction in the control and AF groups. **C**: Expression of ET-1 and CTGF in the control and AF groups detected by immunohistochemistry. **D**: Quantitative analysis of ET-1 and CTGF expression. *P<0.05 *vs* control group.

表2　对照组与AF患者血清ET-1、CTGF和LAD比较

Tab.2　Comparison of SERUM ET-1, CTGF and LAD between the control and AF patients (*Mean±SD*)

Group	*n*	ET-1 (pg/mL)	CTGF (pg/mL)	LAD (mm)
CON	80	21.11±8.34	37.89±15.35	29.61±5.99
AF	138	40.27±13.51**	49.88±12.68**	39.70±7.43**
Z	-	-10.22	-9.85	-8.99
P	-	0.000	0.000	0.000

**P<0.01 *vs* CON.

表3　AF患者血清ET-1、CTGF和LAD比较

Tab.3　Comparison of SERUM ET-1, CTGF and LAD between patients with PaAF and PeAF (*Mean±SD*)

Group	*n*	ET-1 (pg/mL)	CTGF (pg/mL)	LAD (mm)
PaAF	66	33.92±10.90	42.33±10.23	36.70±4.86
PeAF	72	46.09±13.10**	56.79±10.64**	42.44±8.29**
Z	-	-6.43	-7.07	-4.55
P	-	0.000	0.000	0.003

**P<0.01 *vs* PaAF.

响AF的危险因素(P<0.05,P<0.01,表4)。

2.8　临床随访

2.8.1　复发组及未复发组患者基线资料、ET-1和CTGF比较　对行射频消融术的AF患者进行为期6月的随访,共随访100人,失访11人;随访发现复发患者均为PeAF患者,故本实验组将PeAF患者分为复发组(Re)患者(n=16)及未复发组(NRe)患者(n=33),随访结果如下表所示:两组患者年龄、性别、是否合并高血压病、是否合并糖尿病、是否合并脑梗塞、是否口服胺碘酮、是否口服抗凝药、红细胞、白细胞、血小板、BNP、LVEF、ALT、AST、血肌酐及低密度脂蛋白差异均无统计学意义(P>0.05,表5),复发组患者术前、术后血清ET-1和CTGF水平明显高于未复发组,差异有统计学意义(P<0.01,表6)。

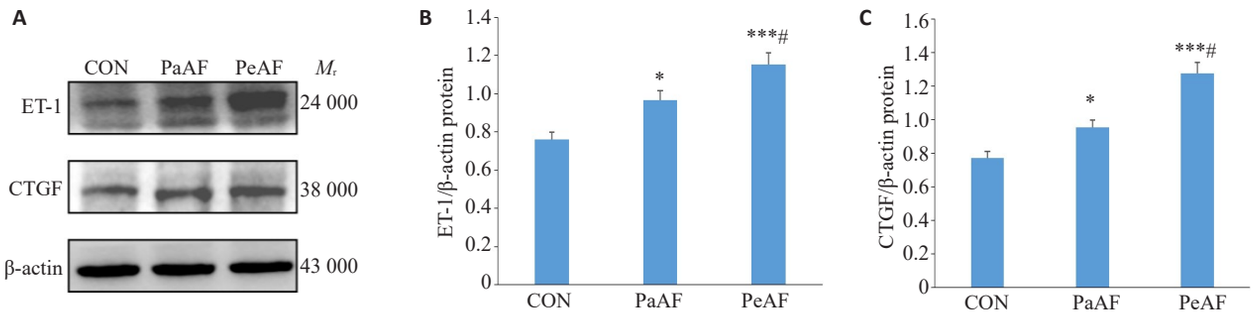

图2 ET-1和CTGF在各组血清单个核细胞中的蛋白含量分析

Fig.2 Western blotting of ET-1 and CTGF expressions in serum mononuclear cells (**A**) and quantitative analysis of their expression levels (**B**, **C**). **P*<0.05 *vs* CON, ****P*<0.001 *vs* CON, #*P*<0.05 *vs* PaAF.

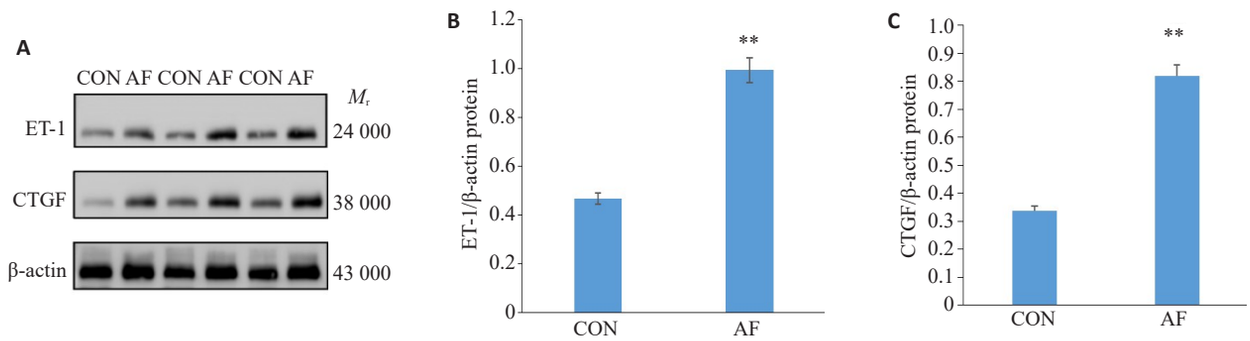

图3 ET-1和CTGF在各组右心耳中的蛋白含量分析

Fig.3 Western blotting of ET-1 and CTGF expressions in the right auricle (**A**) and quantitative analysis of the results (**B**, **C**). ***P*<0.01 *vs* CON.

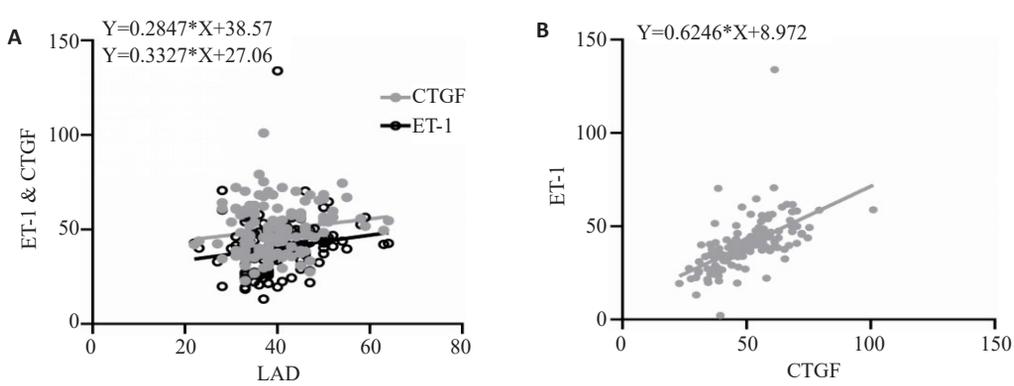

图4 AF患者血清ET-1、CTGF及LAD的相关性

Fig.4 Correlation of ET-1, CTGF and LAD in patients with AF. **A**: Correlation analysis of ET-1 and CTGF expressions with LAD. **B**: Correlation analysis between ET-1 and CTGF expressions.

2.8.2 ET-1、CTGF表达水平与PeAF术后复发的相关性分析及散点图 Pearson相关性分析结果示PeAF患者射频消融术后复发与术前、术后ET-1和CTGF表达水平呈正相关(r_{ET-1}=0.76,r_{CTGF}=0.73,$r_{ET-1'}$=0.77,$r_{CTGF'}$=0.63,*P*<0.01),PeAF患者术前、术后ET-1和CTGF呈正相关(图5)。

2.8.3 PeAF患者术前、术后ET-1和CTGF行logistic回归分析 纳入术前、术后ET-1和CTGF行logistic回归分析,结果显示ET-1、CTGF为影响PeAF复发的独立危险因素(*P*<0.05,表7)。

表4 AF患病风险多因素logistic回归分析

Tab.4 Multivariate logistic regression analysis of the risk factors of AF

Index	β	SE	Wald χ^2	*P*	OR (95% *CI*)
ET-1	0.09	0.03	8.77	<0.00	1.09 (1.03-1.16)
CTGF	0.13	0.04	12.95	<0.00	1.14 (1.06-1.22)
LAD	0.20	0.04	22.88	<0.00	1.23 (1.13-1.33)

表5 复发组与未复发患者基线资料比较

Tab.5 Comparison of baseline data between the recurrence and non-recurrence groups

Ariable	Re (*n*=16)	NRe (*n*=33)	F/χ^2	*P*
Age (year)	63.50±9.89	69.55±12.47	2.87	
Male (*n*, %)	11 (68.75)	20 (60.61)	0.31	
Smoking (*n*, %)	0 (0)	7(21.21)	-	
Hypertension (*n*, %)	8 (50.00)	17 (51.52)	0.1	
Diabetes mellitus (*n*, %)	0 (0)	7 (21.21)	-	
Cerebral infarction (*n*, %)	4 (25)	9 (27.27)	-	
Anticoagulant (*n*, %)	16 (100)	33 (100)	-	
Amiodarone (*n*, %)	16 (100)	33 (100)	-	
RBC/ ($\times10^{12}$/L)	4.40±1.21	4.49±0.76	0.94	*P*>0.05
WBC/ ($\times10^{9}$/L)	6.54±2.03	6.26±2.00	0.22	
PLT/ ($\times10^{9}$/L)	190.06±29.56	208.33±58.62	1.37	
BNP (pg/mL)	70.05±39.11	55.56±33.92	1.78	
LVEF (%)	58.25±6.02	55.36±7.21	0.81	
ALT (U/L)	22.50±9.85	22.24±8.75	0.01	
AST (U/L)	25.50±9.40	28.24±14.48	0.47	
SCR (μmol/L)	68.25±17.73	68.73±19.56	0.01	
LDL (mmol/L)	2.27±0.82	2.48±0.83	0.69	

表6 复发组与未复发组患者术前、术后血清CTGF和ET-1表达情况

Tab.6 Serum CTGF and ET-1 expression before and after operation in patients with and without recurrence

Group	ET-1/(pg/mL)		CTGF/(pg/mL)	
	Before	After	Before	After
Re	56.56±7.81**	25.38±4.96**	67.08±5.24**	31.23±5.04**
NRe	41.68±4.19	17.93±2.43	51.56±7.70	24.55±3.40
t	7.14	5.68	7.27	4.80
P	<0.01	<0.01	<0.01	<0.01

**P<0.01 *vs* NRe.

3 讨论

结构重构和电重构在AF的发生机制中发挥着至关重要的作用，前者主要表现为心肌结构紊乱、心肌纤维化和凋亡等。近年来，研究发现ET-1和CTGF是促纤维化的关键因子[10-13]，且在AF患者外周血和心肌组织中高表达[16, 17]，但是，ET-1、CTGF与不同类型AF的相关性以及联合二者对AF预后的预测价值尚未有明确报道。因此，本研究通过分析AF患者血清和右心耳组织中ET-1和CTGF的表达水平及其与预后的关系，以期对AF预防、诊治提供新的思路。

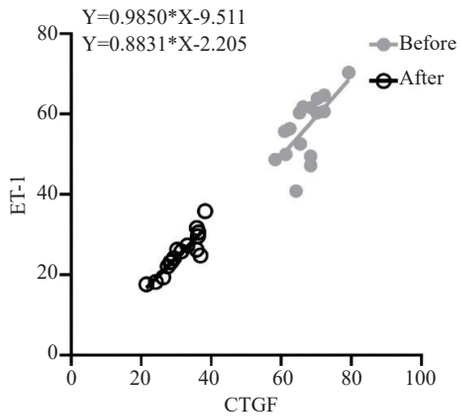

图5 PeAF患者术前、术后ET-1和CTGF的相关性

Fig.5 Correlation between ET-1 and CTGF before and after operation in patients with PeAF.

表7 Logistic回归分析影响PeAF患者射频消融术后复发的独立危险因素

Tab.7 Logistic regression analysis of the independent risk factors for recurrence of PeAF after radiofrequency ablation

Index	β	SE	Wald χ^2	*P*	OR (95% *CI*)
ET-1 (before)	0.43	0.23	3.48	<0.05	1.53(0.98-2.40)
CTGF (before)	0.34	0.16	4.78	<0.05	1.40(1.04-1.90)
ET-1 (after)	0.58	0.21	7.45	<0.01	1.79(1.18-2.71)
CTGF (after)	0.60	0.25	5.70	<0.05	1.82(1.11-2.98)

我们通过对AF患者右心耳组织行HE染色结果显示，CON组心肌细胞排列整齐、致密，肌纤维无断裂、缺损；AF组心肌细胞排列紊乱、间隙增宽，肌纤维断裂明显；Masson染色结果显示，与CON组相比，AF组心肌组织纤维化明显、CVF显著增加，增生的胶原纤维包绕、分隔原有心肌组织。研究发现，CTGF在心脏、肝脏、肺及脉管等组织中呈高表达，并在诱导其组织重构和纤维化中起重要调节作用，尤其与心血管疾病密切相关[18-24]；而且通过CTGF敲除或抑制可阻止纤维化病变的进展而得到进一步证实[25-27]。ELISA和Western blot结果显示，AF组患者血清中CTGF的表达水平显著上升，并且在持续性房颤组中表达水平高于阵发性房颤组。在AF患者右心耳组织中，免疫组化和Western blot结果进一步证实：CTGF的蛋白表达水平显著高于CON组。我们进一步分析发现，AF组患者LAD水平显著高于CON组，且CTGF水平与LAD呈显著正相关。以上结果表明，CTGF表达增加可能参与AF心肌结构改变，并促进AF的发生和进展，与AF不同类型相关。因此，抑制CTGF的表达可能是阻止AF病情进展的新靶点。

ET-1参与血管收缩、组织修复、促纤维化等多种生物学作用[28]。ET-1可使成纤维细胞有丝分裂活性升高，促进成纤维细胞增殖分化，进而诱导纤维化形成[9, 29, 30]。ET-1是转化生长因子β的主要效应因子，促进成纤维细胞和细胞外基质增殖，在心脏中可导致心肌纤维化和肥大[31-33]。研究发现，AF患者外周血ET-1高表达，但ET-1在AF发病中的具体作用目前尚不清楚[34-36]。本研究发现，房颤组患者血清中ET-1表达水平显著升高，且在持续性房颤组内表达水平显著高于阵发性房颤组；Western blot结果进一步得到证实。在对右心耳组织行免疫组化和Western blot检测，ET-1的蛋白表达水平在AF组患者中显著高于CON组。我们进一步分析了Pearson相关性，ET-1与LAD之间呈正相关。以上结果提示ET-1在AF患者中表达显著增加，可能与AF病情进展呈现正相关，并影响心脏结构的改变，我们研究结果表明，ET-1的促心肌纤维化作用可能在AF结构重构中发挥重要作用，进而促进AF的发生发展。因此，抑制ET-1的表达防治策略可能是AF治疗的一个新切入点。

研究证实ET-1可诱导心肌细胞、血管平滑肌细胞、肺成纤维细胞中CTGF表达增加，CTGF介导ET-1在血管平滑肌细胞中的促纤维化作用；在ET-1干预的细胞中，CTGF沉默可消除细胞外基质成分的表达，这表明CTGF不仅直接参与了ET-1诱导的细胞外基质积累，还通过下游效应物参与作用[37, 38]。ET-1和CTGF均为致纤维化因子，且二者关系密切，故本实验联合观察二者在AF中的表达情况，并探讨其与AF不同类型是否存在相关性。在本实验中，我们进一步分析了Pearson相关，结果表明在AF患者中ET-1和CTGF有显著相关性；据此我们联合观察二者的表达水平，在AF组患者血清和右心耳组织中明显高于对照者，这些结果表明，ET-1和CTGF或通过调控心肌细胞的增殖、纤维化促进心房重构，参与AF的发生发展，且在不同类型AF中发挥重要作用。

目前，射频消融术是AF的主要治疗手段之一，即使术中成功达到了所有消融终点，仍然有部分患者术后会出现复发，所以研究AF射频消融术后复发的危险因素具有重要的临床价值。有研究报道，IL-6、CRP、ANP和TIMP-2等的术前表达水平可作为预测AF消融术后复发的独立危险因子[39]。以往对于血清中ET-1和CTGF的表达水平与AF复发率的研究结果大相径庭[40-44]。因此，本研究针对行射频消融术的AF患者进行了6个月的随访[45]，测定患者术前、术后血清中ET-1和CTGF的表达水平，我们发现与未复发组相比，复发组患者基线资料无统计学差异，而术前、术后血清中ET-1和CTGF的水平都明显升高，但二者术后的水平都较术前明显降低。这一结果提示，ET-1、CTGF不仅参与AF的发生发展，而且在AF复发方面也存在相关性，对AF复发有重要的预测价值；而对于术后ET-1和CTGF的低水平，可能与术后服用胺碘酮和抗凝药物有关。并且我们的随访结果发现复发患者AF类型均为PeAF，据此我们进一步行Pearson相关性分析，结果发现ET-1、CTGF与PeAF术后复发呈显著正相关趋势，这进一步表明，ET-1和CTGF的表达水平越高，复发概率越大；并且我们对术前、术后血清ET-1和CTGF行logistic回归分析，结果提示术前、术后二者均为PeAF患者复发的危险因素。综合以上结果提示ET-1和CTGF对PeAF术后复发存在预测价值，即术前、术后ET-1和CTGF的高水平可能预示着PeAF复发的高风险，在这一方面联合二者具有重要的预测价值。

综上所述，在AF患者中ET-1和CTGF表达增高，且与AF的持续时间呈正相关；术前、术后血清ET-1和CTGF水平对PeAF射频消融术后复发有预测价值，这为临床上AF的防诊治提供了新方向。

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