Circulating MicroRNA-34a as a Promising Marker for Early Detection of Heart Failure Diseases in Egyptian Patients

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Abstract.

Heart failure has been considered in the last few years as a major factor in global mortality. Heart failure is known as the reduced ability of the heart muscle to fill or/and to pump adequate blood. Finding a diagnostic biomarker is necessary to help in the early diagnosis of heart diseases. MicroRNAs are new biomarkers that have been used for early diagnosis of various diseases. The work aims to study miR-34a in patients with heart failure and assess its diagnosing role. In this work, a total of (120) subjects were divided into three groups as the following, the control group (n=20) of healthy individuals, group II (n=50) the patients with heart failure post myocardial infarction & group III (n=50) the patients with heart failure without myocardial infarction history. microRNA-34a expression showed high expression and significant elevation (P- value < 0.5) in group-II (31.67 ± 11.8 fold) and group-III (27.31 ± 12.4fold) compared with group-I (1.47 ± 0.7 fold). Our results showed that microRNA-34a plays a critical role in patients with heart failure disease and can be used as a biomarker for the diagnosis of early stages of heart failure disease.

Keywords: Heart Failure; miR-34a, Biomarker.

Introduction

Heart failure (HF) is a complex clinical disease characterized by inadequate cardiac output caused by myocardial damage. HF is still the major cause of morbidity and mortality worldwide (1). The most frequent type of heart failure is chronic heart failure (CHF). While hypertension, coronary heart disease, and diabetes mellitus are the most common causes of CHF, genetic association studies have demonstrated that genetic predispositions have a significant role in the risk and prognosis of CHF (2).

The word "biomarker" (from "biological marker") refers to "an assessable and quantifiable biological marker used to evaluate patient health and physiology about disease risk and diagnosis." In 1989, the phrase was coined to mean 'statistical parameter' (3). Even though many distinct transcription factors and signaling molecules are involved in this pathogenic process (4), mounting

data suggests that a significant number of miRNAs contribute to heart aging and HF (5).

After examination of many molecules, only "B-type natriuretic peptide (BNP)" and "N-terminal pro-B-type natriuretic peptide (N-terminal pro-B-type natriuretic peptide)" come close to the features of "ideal" HF biomarkers and are frequently "regarded as the reference standard against" which other prospective biomarkers must be evaluated. "Both BNP and pro-BNP" are key outcome predictors in chronic HF (6). Heart failure diagnosis/prognosis is frequently challenging, and biomarkers, in conjunction with other clinical tests, can help.

Interleukins (IL), a type of cytokine, play an important role in the initiation of "immunological reactions such as inflammation". Once an IL is produced, it goes to its target cell and binds to it via "a receptor molecule" on the cell's surface. This contact initiates a cascade of signals within the target cell, which eventually changes the cell's behavior (7). Interleukin-18 is a pro-inflammatory cytokine that is structurally identical to IL-1 and belongs to the IL-1 superfamily. IL-18 stimulates the production of interferon-gamma (IFN-G), which causes a robust Th1 response (8).

MicroRNAs (miRNAs) are noncoding RNAs with a length of about 22 nucleotides that play a role in gene regulation. Mature miRNAs have been found in over 3000 different species, spanning from plants to humans (9). A miRNA's conventional mode of action is to suppress gene expression by "binding to complementary sequences (6-8 nucleotides) inside the 3′ UTR of its target mRNAs". Because of "this partial complementarity", the expression of a miRNA's target is inhibited via destruction or repression of translation of the attached mRNAs (10). MiRNA expression profiles change between cell types, and cell/tissue/organ-specific miRNAs (or profiles) may suggest distinct illnesses. MiRNAs in the circulation are either actively secreted by living cells or passively released as a result of cell death (11).

A few numbers of miRNAs have been thoroughly described with known targets in cardiac illness, implying their potential to serve as biomarkers for HF. MiR-1, the most abundant miRNA in heart tissue, targets the important "cardiac-specific transcription factors Hand2 and Irx-5", both of which are required for "cardiogenesis and cardiac conduction" (12). A recent study found that miRNA-based medicines could effectively cure HF in a large animal (pig) model (13).

MicroRNA-34a (miR-34a) is found on "chromosome 1p36.23" and is mostly expressed in "adipocytes, macrophages, endothelial cells, smooth muscles" (14; 15). Meanwhile, miR-34a overexpression caused endothelial cell senescence, which is crucial in atherosclerosis (16). Several investigations have identified miR-34a as a key modulator of aging-related heart dysfunction via altering sirtuin-1 expression (17, 18, 19).

This study aims to determine the role of miR-34a in the progression of heart failure disease and its ability to serve as a biomarker for early detection of HFDs in Egyptian patients with heart failure diseases.

Subjects.

Written informed consent was obtained from all subjects. The study was conducted between December 2021 and February 2022 on 120 subjects. Patients of both genders (male and female) and between 45 and 65 years of age were included. Subjects were grouped as follows:

The first group (control) is 20 healthy volunteers.

The second group (HFpMI) is 50 heart failure patients post-MI.

The third group (HFwMI) is 50 heart failure patients without MI history.

Inclusion Criteria: Patients diagnosed with recent HFD (within the period between three to six months post the diagnosis of heart failure) post-MI (group II) or have one or more risk factors for HF including

diabetes, obesity, hypertension and hyperlipidemia without MI history, rejection fraction for all patients under study was less than 35%.

Exclusion Criteria: Severe respiratory diseases, liver, and kidney failure, and malignancies.

All candidates are subjected to:

-Full history taking

- Physical examination including measurement of height, weight, BMI, and blood pressure.

-Laboratory investigations including: GPT, creatinine, HbA1c, lipid profile, IL-18, hsCRP & proBNP.

Methods

Creatinine, GPT & lipid profile (total cholesterol, triglycerides, HDL, LDL, and VLDL) were assayed using "RANDOX kit (RANDOX Laboratories LTD, UK)" according to the manufacturer's guidelines, then were measured by using (DIALAB DTN-405 Chemistry Analyzer, Austria).

HBA1C was determined using (Bioscien, ARENA, turbidimetric kit, Egypt), then had been measured by using (DIALAB DTN-405 Chemistry Analyzer, Austria).

hs-CRP, IL-18 & NT pro-BNP were determined by using ELIZA kits "The Eagle Biosciences, INC, Nashua, NH kit", "The Quantikine™ Human Total IL-18/IL-1F4 Immunoassay, USA R&D Systems, Inc. kit ", "FINE TEST COMPANY, Wuhan, China kit " and "KAMIYA BIOMEDICAL COMPANY, USA kit " respectively, according to the manufacturer's guidelines by using the microplate reader (infinite f50, TECAN, Austria, GmbH) and plot software (Curve Expert 1.4).

Real-time reverse transcription (RT)-PCR method was used to detect and measure miRNA-34a in serum samples. Total RNA, including miRNAs, was isolated from serum using the "miRNeasy Mini Kit (QIAGEN, Clinilab, Egypt)" and the "miScript® II RTKit (QIAGEN, Clinilab, Egypt)" according to the manufacturer's guidelines. cDNA synthesis was performed according to the manufacturer's

instructions. To quantify miR-34a cDNA, we used the primer pair according to (20) for cDNA amplification. The miScript primer sequences used in this study were synthesized at "QIAGEN, Sample & Assay Technologies, Germany "The sequence of miR-34a-3P is CAAUCAGCAAGUAUACUGCCCU. The number of bases of miScript primer is 22mer. Real-time PCR was performed using an applied biosystems ViiA™ 7 system. Amplify miR-34a cDNA using 2x QuantiTect SYBR Green PCR Master Mix and RT-PCR Reagent Kit.

Non-template controls were used to check the contamination of the cDNA template, which was prepared by adding the appropriate volume of PCR-grade sterile water to the SYBER Green PCR master mix. The internal control gene, non-coding small RNA SNORD68, was used according to the applied biosystems application note. The relative quantitative (RQ) of miR-34a in the studied subjects was calculated using the non-coding small RNA SNORD68 as an internal control. The point at which the amplification plot crossed the threshold was defined as threshold cycle CT; ΔCT was determined by subtraction CT that of SNORD68 (calibrator) from CT of target miR-34a; $\Delta\Delta$CT was calculated by subtraction (ΔCT) of control subjects from (ΔCT) of target miR-34a; and finally, relative quantitative (fold) of miR-34a = $2^{(-\Delta\Delta ct)}$.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare different groups. The statistical analysis includes the arithmetic mean value, standard deviation, hypothesis student "t" test, Pearson correlation "r", and the significance of the outcome (P). P-values less than 0.05 were considered statistically significant. The sensitivity and specificity were determined using receiver-operating characteristic (ROC) curve analysis. Statistical software (SPSS, Inc., Chicago, USA, version 26) was used to carry up all statistical analyses (21).

Results

This study showed that there was no significant difference in age or gender among all studied groups. The level of ALT was within the normal range in all groups studied; however, the percentage of male patients with HF was more than female patients within the three groups but not statistically significant P-value **> 0.05**. As indicated in Table 1, the findings of this study revealed a statistically significant rise in the mean values of HbA1c, Creatinine, total cholesterol, triglycerides, LDL, and VLDL in groups II&III when compared with group I (control). In addition, when compared to the control group, proBNP levels in the study groups II & -III increased significantly. Table 2 shows that the studied group II & group-III had significantly higher levels of hs-CRP and IL-18 when compared to the control group.

As regards the result of miR34-a, the mean serum miR34-a levels were considerably significantly higher in group II (HFpMI) and group III (HFwMI) than in the control group, miR34-a was a more significant increase in group II (HFpMI) than group III (HFwMI) with P-value **<0.001**, as shown in Figure 1 and Table 3.

As regards data obtained by ROC curve analysis in patients with HF post-MI, miR34-a at a cut-off level >11.5 ng/ml had a diagnostic sensitivity of 0.80, specificity 1.0, with "an area under the curve" (AUC) 0.891, table 4, figure 2, while in patients with HF without MI history, cut off level >15.3 of miR34-a had a diagnostic sensitivity of 0.86, specificity 1.0 and AUC 0.983, table 5, figure 3.

As shown in Tables 6&7 there was no correlation between miR-34a expression and different parameters under study in groups II & III.

Table 1. Comparison between studied groups

Parameter	Group I (n= 20)	Group II (n= 50)	Group III (n= 50)	P-value
SBP(mmHg)	118 ±14.3	125.667	129±18.3	
DBP (mmHg)	81 ±6.3	82.333	89±11.2	< 0.05
Age	56.50 ±6.50	56.48 ±6.0	57.5 ±6.4	> 0.05
Sex (male/female)	13/7	35/15	30/20	> 0.05
ALT (U/L)	23.2 ±6.0	29.02 ±6.8	28.78 ±6.3	> 0.05
HBA1C (%)	4.78 ±0.5	6.93 ±2.2	6.4 ±2.2	
Creat. (mg/dl)	0.74 ±0.2	1.82 ±0.6	2.25 ±0.7	< 0.05
Chol. (mg/dl)	139.85 ±18.0	212.9 ±40.6	171.8 ±45.2	
Trigly. (mg/dl)	76.8 ±24.7	150.38 ±55.8	115.38 ±35.8	
HDL (mg/dl)	60.75 ±11.0	39.66 ±9.7	39.74 ±9.7	<0.05
LDL (mg/dl)	63.74 ±16.7	143.16 ±40.9	109.0 ±47.1	
VLDL (mg/dl)	15.36 ±4.9	30.07 ±11.2	23.07 ±7.2	
proPNP(pg/ml)	58.38 ±19.84	942.26 ±407.34	927.54 ±356.322	< 0.05

Table 2 Comparison between studied groups according to inflammatory markers

Parameter	Group I (n= 20)	Group II (n= 50)	Group III (n= 50)	P-value
hs-CRP (mg/L)	2.2 ±0.9	17.54 ±6.0	21.19 ±8.0	< 0.05
IL18 (ng/ml)	3.11 ±0.5	25.64 ±13.8	18.44 ±6.8	

Table 3: Serum Micro RNA- 34a level in control and other studied groups.

Parameter	Group I (n= 20)		Group II (n= 50)		Group III (n= 50)		P-value
	Mean	SD	Mean	SD	Mean	SD	
miR-34a (fold)	1.47	0.7	31.67	11.8	27.31	12.4	< 0.05

Table 4: ROC Curve analysis for heart failure patient's post-myocardial infarction (group II).

Test Result Variable(s)	Cut off	AUC	sensitivity	Specificity	PPV	NPV	Asymptotic Sig.[b]	95% CI	
								Lower	Upper
miR-34a (fold)	11.5	0.891	0.80	1.0	100	83.3	0.000	0.818	0.965

Table 5: ROC Curve analysis for heart failure patients without myocardial infarction history (group III).

Test Result Variable (s)	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	Asymptotic Sig.[b]	95% CI	
								Lower	Upper
miR-34a (fold)	15.3	0.983	0.86	1.0	100	87.7	0.000	0.960	1.0

Table 6: Correlation between miR-34a and different parameters under study in group II.

Parameters		HBA1C (%)	hsCRP (mg/L)	IL-18 (ng/ml)	LDL (mg/dl)
miR-34a (fold)	R	0.194	-0.135	-0.105	-0.021
	P	0.177	0.351	0.469	0.884

Table 7: Correlation between miR-34a and different parameters under study in group III.

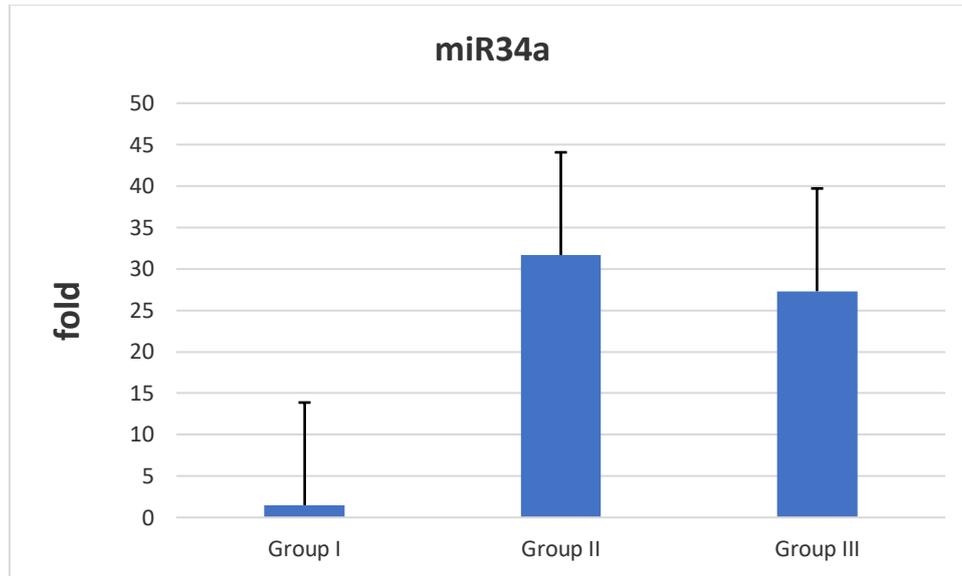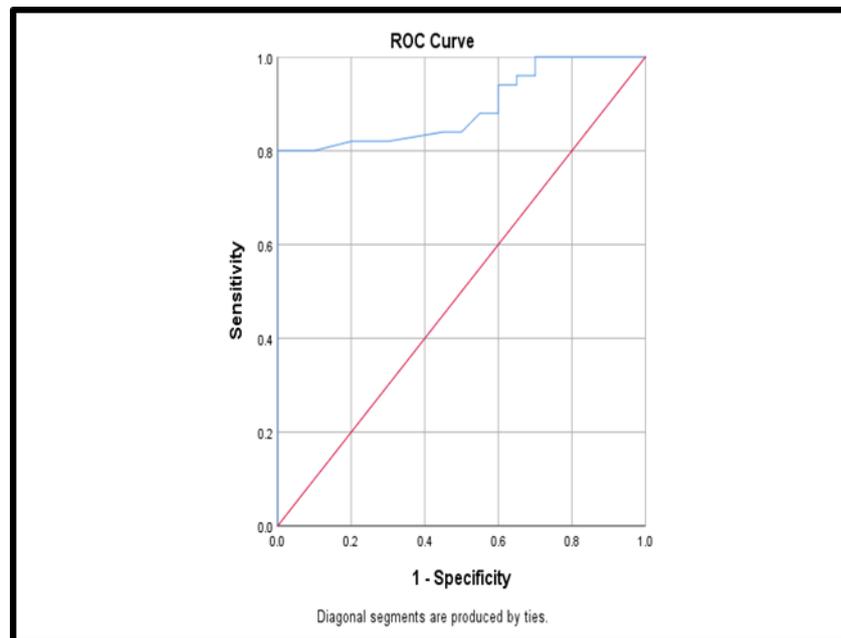| Parameters		HBA1C (%)	hsCRP (mg/L)	IL-18 (ng/ml)	LDL (mg/dl)
miR-34a (fold)	r	-0.065	-0.418	-0.105	0.146
	p	0.655	0.003	0.466	0.311

Fig. 1: miR-34a of control and other studied groups

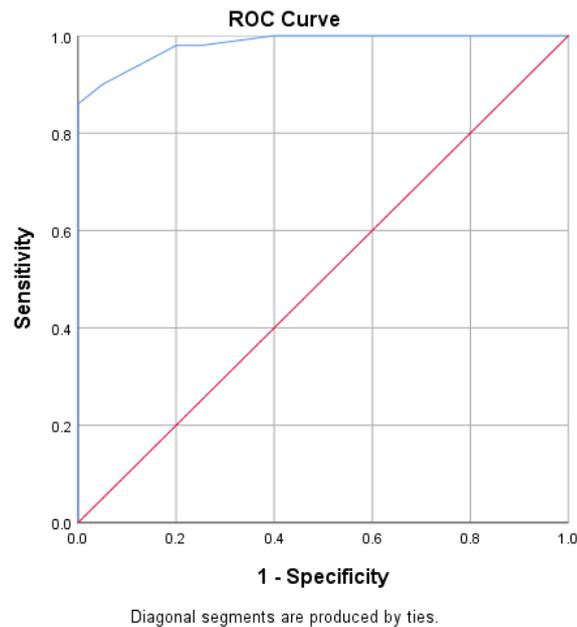**Fig. 2: ROC curve for miR-34a to diagnose heart failure disease post-myocardial infarction.**

Fig. 3: ROC curve for miR-34a to diagnose heart failure disease in patients without myocardial infarction history.

Discussion

Several scientific papers have suggested possible links between changes in serum or plasma microRNA (miRNA) expression and the prevalence of cardiovascular diseases (CVD) such as coronary heart disease, atherosclerosis, acute coronary syndromes, coronary artery disease, and heart failure. The goal of this research is to help disclose the expanding contemporary importance of miRNAs, particularly in heart failure, and to suggest beneficial miRNAs that can be discovered in blood serum or plasma and have the potential to become diagnostic or prognostic biomarkers. Further research into the hypothesized miRNAs should be conducted.

Several biomarkers, including NT-proBNP and troponin T, have been reported to predict cardiovascular evidence after AMI; however, whether such biomarkers can predict future HF in post-AMI patients is unknown (22). As a result, identifying biomarkers that predict the risk of HF progression in post-AMI patients is critical for optimizing management and therapy regimens.

Nonetheless, the genetic basis for sporadic CHF is poorly understood, and the discovery of a novel molecular biomarker of CHF may aid in the early detection and treatment of this disease.

Circulating miRNAs have shown considerable potential as a novel class of diagnostic or prognostic biomarkers for predicting cardiovascular illnesses such as acute myocardial infarction and heart failure throughout the last decade. This is mostly due to their ability to distinguish between normal and sick cells (23). At the same time, their increased stability in the circulation and fixed tissues compared to proteins and other nucleic acids makes them particularly well-suited to sample and analysis (23). The current work concentrated on a single miRNA family, the miR-34 family, and the miR-34a3P variant. The justification for selecting miR-34a as a main target of our inquiry was based on the following evidence: (i) miR-34a expression is increased in cardiac tissue from patients with heart disease or cardiac injury (24); (ii) miR-34a cardiac expression is also increased in a mouse model of MI

(25); and (iii) inhibition of miR-34a via anti-miR-34a therapy improves reverses cardiac remodeling and cardiac function in mouse models of MI or heart hypertrophy (26). Our study has shown a high expression of miR-34a (30-fold) in HFpMI patients and (27-fold) in HFwMI patients when compared with the healthy individuals (control group), these results highlighted the role of miR-34a in HFD.

Matsumoto et al. showed that plasma levels of miR-34a in circulating blood of post-acute myocardial infarction (AMI) patients with HF were substantially linked with left ventricular ejection fraction (LVEF) (27), which is consistent with our findings. In addition, it has been demonstrated that miR-34 family members are increased in the cells of the heart in response to AMI, and systemic injection of anti-miR-34 meaningfully inhibits heart dysfunction and remodeling in a mouse model of AMI.(28)

Furthermore, a study found that miR-208b & miR-34a levels serve as predictors of LV remodeling following AMI, with a relationship to 6-month mortality or the development of HF (29).

In the present study, we reported a positive correlation between serum creatinine level in HF patients under study with Pro-BNP level, additionally, when we compared the serum creatinine level of patient groups with the control group, there was a significant increase. These findings suggest the presence of a relationship between renal impairment and heart failure disease.

Creatinine is routinely used in clinical practice to assess the effects of HF medications, notably diuretics, which agrees with our findings. (30).

Other studies back with our findings. Cole RT et al. (31) found that twenty percent to fifty percent of individuals with chronic, stable HF and thirty to sixty percent of patients with abruptly decompensated HF had some degree of renal impairment.

The study indicated a significant increase in hs-CRP levels in patients when compared to controls, which is consistent with prior studies (32; 33). This rise in CRP levels could be attributed to the fact that CRP binds to LDL particles in atherosclerotic plaques, causing complement activation and therefore being pro-inflammatory and contributing to atherogenesis. Furthermore, significant amounts of hs-CRP, LDL-C, and HbA1C expression were discovered, which is consistent with previous research.(35 ;34)

In our investigation, it was discovered that the diabetic patients with HF illness categories were substantially higher than the control group. This suggested that hyperglycemia is a risk factor for atherosclerosis and may play a role in the development of HFD. This is consistent with the findings of Esraa et al., who demonstrated that hyperglycemia is a risk factor for atherosclerotic coronary artery disease (36); additionally, Stephen et al. reported that diabetes is associated with more extensive atherosclerosis, inadequate compensatory remodeling, and HFD (37).

In agreement with Li H. et al., (38), we reported that significant increase in total cholesterol, triglycerides, low-density lipoprotein (LDLc), and very low-density lipoprotein (VLDL) in patient serum within group II (HFpMI) and group III (HFwMI), but high-density lipoprotein (HDL) level was within the normal level in studied groups when compared with control group. These findings reflect the effect of lipid profile levels on CHF.

Furthermore, in this work, ROC analysis revealed that miR-34a (AUC 0.891) (sensitivity 0.80) in patients with HF post-MI, while (AUC 0.983) (sensitivity 0.86) in patients with HF without MI history. These findings, which agreed with Li H. et al., (38), suggested that miR-34a may be employed as a marker of HFDs.

Conclusions

The current investigation found that participants with high levels of pro-BNP had significantly increased levels of miR-34a (fold) (P- value 0.05). We reported that serum miR-34a can act as an early predictor biomarker, which is useful in preventive medicine.

Recommendations:

More other studies on a greater number of subjects with more and better statistical analysis should be done.

Further studies to determine the role of miR-34a in other cardiac diseases rather than heart failure are needed.

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