The Importance of autophagy, microtubules and microtubule inhibition in patients with heart failure with reduced ejection fraction

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BACKGROUND AND AIM

In this study, we aimed to compare the serum beclin-1 levels which is one of markers and moderators of autophagic activity and the β1 -tubulin level, which is one of the cardiomyocyte structure proteins in the serum of patients with heart failure with reduced ejection fraction (HFrEF), and those healthy subjects. Also, we investigated serum beclin-1 and β1 -tubulin levels according to etiological classifications (ischemic/non-ischemic subgroups). Additionally, the subset of patients using colchicine as a microtubule inhibitor for at least three months due to HFrEF was included

METHODS

This study included 50 patients with HFrEF (25 with ischemic etiology, 25 with non-ischemic etiology) and 30 healthy subjects between January 2018 and December 2019 in Istanbul University Cardiology Institute. Serum beclin-1 and β1 -tubulin levels were determined by using ELISA method by the ELISA Kit.

RESULTS

Although serum beclin-1 and β1-tubulin levels of all HFrEF group did not reach statistical significance compared to the control group, serum beclin-1 and β1-tubulin levels were increased (p: 0.64) ((p: 0.6) respectively.). However, NT-proBNP levels were found significantly higher (p:0.01) . .Serum beclin-1 and serum β1-Tubulin levels correlated with ejection fraction in 50 patient with HFrEF (p: 0.018, R^2: 0.088). (p: 0.018, R^2: 0.086). In the non-ischemic etiology subgroup with HFrEF especially had higher serum beclin-1 levels (p:0.01).There was also no significant correlation between creatinine and eGFR levels and autophagic activity (p:0.482). Also, we found lower levels of NT-proBNP that did not reach statistical significance and higher beclin-1 levels to reach statistical significance (p: 0.015) in the colchicine using patient subgroup(Table 1).

On the contrary, β1-tubulin levels were increased in ischemic HF patients compared to the non-ischemic subgroup (p: 0.26). In addition, a decrease non-significant in β1-tubulin (p=0.29) and NT*proBNP (p=0.69) levels were found in the subset analysis (n=13) of non-ischemic HF patients using colchicine. However, the subgroup of patients receiving low-dose colchicine for at least three months had better EF (p=0.009 and smaller diastolic left ventricular diameters (p=0.002), respectively(Table 1).

DİSCUSSİON

The autophagic response has been described in various pathophysiological situations, including neurobiology, cancer, cardiovascular disease, and infectious diseases [1-4]. However, their roles in diseases were not fully understood as they can act in different conditions to work towards cell survival or induce cell death.As the mammalian ortholog of the yeast Atg6 gene, beclin-1 is an essential mediator of autophagy [5,6,7,8,9,10]. Beclin-1 forms a multimeric complex with vacuolar protein sorting 34 (Vps34) and class 3 phosphatidylinositol 3-kinase (PI3k), which is necessary for the formation of autophagosome. Component of various PI(3)K complexes beclin-1 interacts with PIK3C3/VPS34 to signal the onset of autophagy. Atg6/beclin-1 Inhibited by binding to Bcl2 [5,6,7,8,9,10](Figure 1).

Pharmacological agents that improve myocardial performance can be described by the framework: calcitropes alter intracellular calcium concentrations; myotropes affect the molecular motor and scaffolding; and mitotropes influence energetics(11). Traditional inotropic agents—including catecholamines, phosphodiesterase-3 inhibitors, sodium-potassium adenosine triphosphatase (ATPase) inhibitors, and mixed-mechanism calcium sensitizers and phosphodiesterase-3 inhibitors modulate calcium signaling in the myocardium but are associated with poor long-term outcomes. Microtubules play crucial roles achieved by their decoration with proteins/enzymes as well as by posttranslational modifications. G-actin (actin) between the alpha, beta-tubulin complex (microtubules) at which the end will remain in an equilibrium state with no net growth or shrinkage is the critical concentration. Colchicine Activates Actin Polymerization by Microtubule Depolymerization.It is known that two cytoskeleton components, microtubules, and actins filaments, are required for efficient endocytosis. An important property of actin is its ability to produce movement in the absence of motor proteins(12)(Figure 2).

CONCLUSIONS

- **Autophagy especially increased in the HFrEF with non-ischemic etiology and patients used colchicine subgroup. However, β1- tubulin levels had increased in the ischemic HFrEF patients according to the non-ischemic and decreased the patients who used the colchicine subgroup. Therefore, low dose colchicine probably regulates autophagy, microtubules inhibition, and vesicle trafficking in HFrEF.**
- **Also, our study gives the impression that the adverse remodeling of the left ventricle can be reversed by low-dose colchicine thanks to autophagy.In addition, actin microfilaments are more effective in the microenvironment,and due to the inhibition of microtubules, they bind more to myosin heads (myotropes effect) such as omecamtiv mecarbil.**
- **Again In this context, colchicine can also prevent covid-19 and atherosclerosis-related endotheliopathies with its anti-inflammatory and cytokine storm inhibitory effects.**

LIMITATIONS

- **Limitations of the study were the nonrandomized prospective cross-sectional observational design, relatively small number of patients.**
- **It was investigated whether there is a correlation between serum beta-tubulin-1 and Beclin-1 levels and beta-tubulin-1 and Beclin-1 levels.**
- **The study was not performed on cardiac biopsy material.**

Keywords:Heart failure • Dilated cardiomyophathy • Cell death • Autophagy • Beclin-1,microtubules* β1- tubulin

Parameters A	Patient(N=50)	Control(N=30)	*P -Value*
NT-proBNP(pg/dl)	2230.4±2079.7	53.6±21.5	*0.01*
Beta-Tubulin(pg/dl)	97.4±174.8	81.3±172.7	*0.6*
Parameters B	Ischemic (N: 25)	Non-ischemic (N: 25)	*P -Value*
NT-proBNP(pg/dl)	2647±2182	1813±1924	0.01
Beta-Tubulin(pg/dl)	108±222	53±95	0,26
Parameters C	Colchicine + (N: 13) (values after treatment with colchicine for at least three months)	Colchicine – (N:37)	*P -Value*
NT-proBNP(pg/dl)	*2029.9±2344*	*2300±2000*	*0.69*
BETA-Tubulin(pg/dl)	*37±81*	*97±194*	*0.29*
EF(%)	*32.6±6.4*	*27.5±3.2*	*0.009*
LVDD(mm)	*58.1±8.2*	*66.1±9.0*	*0.002*
TAPSE(mm)	*18.4±3.0*	*19.8±2.7*	*0.33*
Parameters of D Group	All Patients (N: 50)	Controls (N: 30)	P-value*
EF (%)	31.3±6.2	60	0.001
NT-proBNP (pg/dl)	2230.4±2079,7	53.6±21,5	0.01
Beclin-1 (ng/ml)	6.1±10.4	2.7±6.3	0.64
Parameters of E Group	İschemic HFrEF subgroup(N:25)	Non-ischemic HFrEF subgroup (N: 25)	P- value
Age	64.4±10,2	53.9±13.3	0.004
Gender (male %)	23 (%92)	20 (80%)	0.2
Diabetes Mellitus (%)	14 (%56)	8 (32%)	0.08
Hypertension (%)	19 (76%)	10 (40%)	0.01
Hyperlipidemia (%)	19 (76%)	6 (24%)	0.0001
Cigaret (%)	18 (72%)	6 (24%)	0.001
Alcohol (%)	1 (4%)	2 (8%)	0.5
NT-proBNP (pg/dl)	2647±2182	1813±1924	0.01
Beclin-1 (ng/ml)	2.07±4.7	12.7±16.1	0.01
Parameters of F Group	Colchicine + HFrEF subgroup (N: 13)	Colchicine – HFrEF subgroup (N:37)	P Value
EF (%)(Initial)	27.5±3.2	32.6±6.4	0.009
LVD (mm) 69±7.6(Initial)		59.7±8.9	0.002
LA (mm) (Initial)	46.8±9.4	46.0±8.8	0.59
RVD (mm)	25.0±1.8	25.5±3.3	0.54
TAPSE (mm) (Initial)	18.4±3.0	19.8±2.7	0.33
NT-proBNP (pg/dl) (3 months later)	2029.9±2344	2300±2000	0.69
Beclin-1 (ng/ml) (3 months later)	12.44±10	3.4±8.4	0.015

The importance of autophagy, microtubules and microtubule inhibition in patients with heart failure with reduced ejection fraction.

Table 1.

A) Average of ejection fraction(EF) values, NT-ProBNP and Beta-tubulin levels of patients with total Heart Failure reduced EF(HFrEF) (N: 50) and Control Group (N: 30) Participating in the Study(mean ± SD)(P:0.01;P:0.29 respectively)..

B) NT-proBNP and Beta-tubulin values of HFrEF patients according to ischemic (N: 25) and non-ischemic (N: 25) etiology patient groups included in the study(mean ± SD)(0.01;0.26 respectively)

C) Differences in NT-ProBNP and Beta-tubulin levels and echocardiographic parameters of for at least three months colchicine treated non-ischemic subgroup(N:13) and not using colchicine patient group in the HFrEF

patient group (N: 50) included in the study (mean ± SD)(0.69;0.29;0.009;0.002 respectively).
****Continuous variables are presented as mean ± SD and dichotomous variables as percentages. NS, not significant. A two-tailed t-test was used for comparison of means, and x2-test for percentages.***
D) Average of Echocardiographic EF values, NT-ProBNP and Beclin-1 Levels of Patients with Heart Failure (N: 50) and Control Group (N: 30) participating in the study(mean ± SD).
E) Differences in NT-proBNP and Beclin-1 values of patients with heart failure according to ischemic (N: 25) and non-ischemic (N: 25) etiology included in the study(mean ± SD)..
F) Differences in NT-ProBNP and Beclin-1 Levels and echocardiographic parameters of colchicine used(N:13) and non-colchicine used patients in the HFrEF patient group (N: 37) included in the study (mean ± SD).
****Continuous variables are presented as mean ± SD and dichotomous variables as percentages. A two-tailed t-test***

B) was used for comparison of means, and x2-test for percentages.

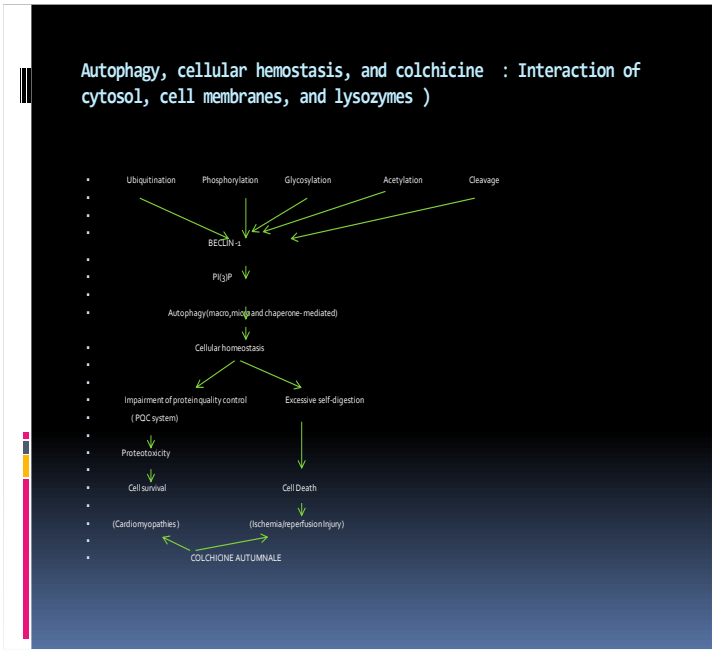

Figure 1.
Autophagy,cellular hemeostasis,and colchicine:interaction of cytosol,cell membrane,and lysozymes

Figure 2. Post-translational modifications of Beclin 1 affect protein stability, confirmation, activity, and its interactome and can be used as a molecular rheostat to fine-tune autophagic activity.

•Targeting Beclin 1 modifiers to regulate Beclin 1 post-translational modifications could provide a possible therapeutic intervention for upregulatin autophagy.

•Colchicine accelerates the physiological clearance of misfolded proteins from cells through the protein quality control (PQC) system and cytoprotective autophagy.

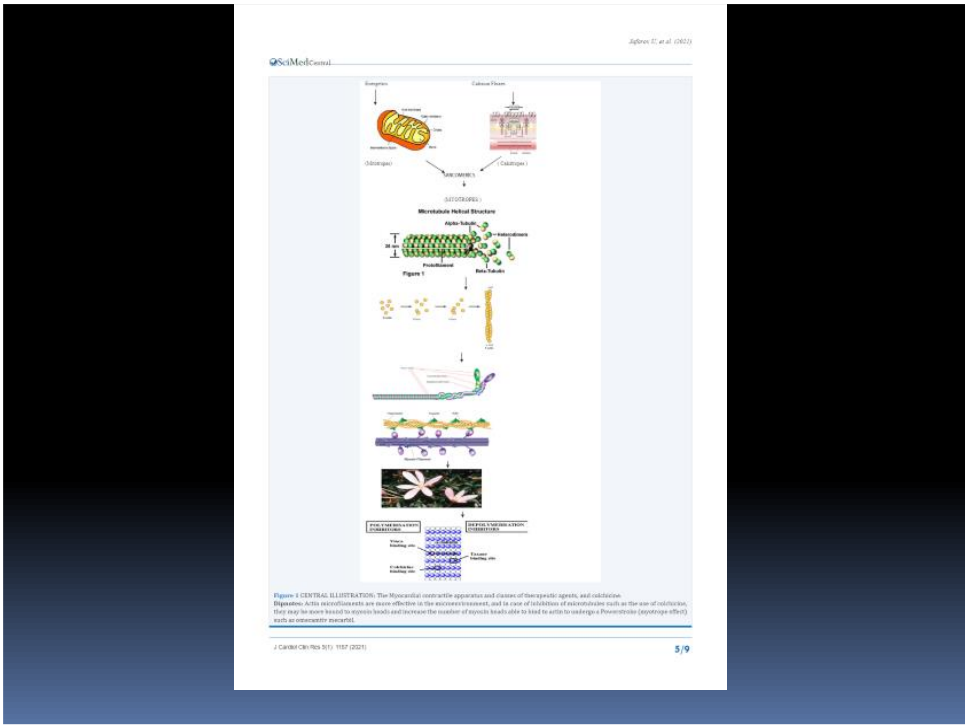

Figure 2.

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