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Research Article

Evaluation of the Importance of Microtubules and Microtubule Inhibition through blood β1- Tubulin Levels in Patients with Heart Failure with Reduced Ejection Fraction

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Keywords

- Heart Failure
- β1- tubulin
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Abstract

Background and Aim: Beta-Tubulin is a microtubule that binds with α-Tubulin to form tubulin heterodimer which is a structural protein of cardiomyocytes. Colchicine inhibits tubulin polymerization.The aim of our study is to determine the β1 -tubulin level, which is one of the cardiomyocyte structure proteins in serum and to investigate the relationship with heart failure, and also to investigate the importance of microtubule inhibition via β1 -tubulin- levels,in stage 4 heart failure with reduced ejection fraction (HFrEF).

Methods: We investigated serum β1- tubulin levels according to etiological classifications (ischemic/non-ischemic subgroups). Also, the subgroup with 13 non-ischemic HFrEF patients using low dose colchicine (0.5-1mg), as a microtubule inhibitor for at least three months was also included. Blood samples have been centrifuged and β1- tubulin plasma concentrations were measured by the Elisa method with Human β1- tubulin Chain Elisa kit.

Results: β1- tubulin levels had increased in ischemic HFrEF patients according to the non-ischemic subgroup (p:0.26). Besides, in the subgroup analysis of non-ischemic HFrEF patients used colchicine (n=13), was detected decreased levels of β1- tubulin (p=0.29), and NT*proBNP (p=0.69). But, for at least three months low dose colchicine used patient subgroup had better EF (p=0.009), and smaller diastolic left ventricular diameters (p=0.002), respectively.

Conclusions: Our study gives the impression that the adverse remodeling of the left ventricle can be reversed by low dose colchicine.Thus, actin microfilaments are more effective in the microenvironment, and due to the inhibition of microtubules, they bind more to myosin heads (myotropes effect).

INTRODUCTION

The 3 broad components of the myocardial machinery are the contractile elements and regulatory proteins in the sarcomere, the calcium ion (Ca^{2+}), cycling elements in the cell and sarcoplasmic reticulum membranes, and the energetic elements, including adenosine triphosphate (ATP), produced by the mitochondria. 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 [1]. 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 [2].

Actin is a widely expressed protein found in almost all eukaryotic cells. Actin is a globular protein (G-actin), that assembles into filaments (F-actin), and is important for cell movement, intracellular movement, muscle contraction, and many other functions. Cooperative activation of actin-myosin interaction by tropomyosin (Tm), is central to the regulation of contraction in muscle cells and cellular and intracellular movements in nonmuscle cells. Actin is a promiscuous protein, interacting with many other proteins [3], and is also subject to many different post-translational modifications [4]. There are six actin genes in the human genome in connection with actin isoforms [1]. Actin–myosin interaction and force generation are key to myocardial function and central to the pathophysiology of heart failure [1,4-6].

Microtubule is a much more complex molecule. The building block of this protein is a dimer called tubulin, which is composed of two subunits: α-tubulin and β-tubulin. α-tubulin

and β- tubulin form a filamentous chain called "protofilament". Microtubules are built by arranging 13 such protofilaments around an empty core. This gives rise to a tube-like construction (hence the name microtubule), which is stiffer, longer and wider than actin. Microtubules have a distinct organizing site called the "centrosome". Microtubule polymerization begins at this organelle. The end where faster polymerization occurs is called the plus terminus. The end where slower polymerization takes place is called the minus end. Microtubules grow from the centrosome towards the membrane, by anchoring their minus end to the organelle. Once microtubules reach the membrane they detach from the centrosome and create a highly dynamic network. The formation of this network is assisted by a group of proteins with microtubule-binding domains called Microtubule Associated Proteins (MAP). Also, kinesin motor proteins that simultaneously bind two microtubules aid in remodeling cytoskeletal networks by sliding microtubules along one another. Microtubules can slide together or apart depending on filament organization and kinesin directionality. Free microtubules, sliding on suspended microtubules, not only rotate around their own axis but also move around the suspended microtubules with right-handed helical trajectories [7-9].

The mechanism of action of colchicine is through the inhibition of tubulin polymerization and potentially also through effects on cellular adhesion molecules and inflammatory chemokines. Also, colchicine modulates regulation of the protein quality control (PQC), system via chaperone-mediated autophagy and post-translational modification and also by effective mitochondrial dynamics and homeostasis. These modifications have a role in multiple cellular functions, ranging from cell motility, cell cycle progression, or cell differentiation to intracellular trafficking and signaling [10-13].

All cytoskeletal network proteins such as actin, microtubules, and intermediate filaments interact directly with each other [7]. Actin microfilaments are more effective in the microenvironment, and in case of inhibition of microtubules, they may be more bound to myosin heads and increase the number of myosin heads able to bind to actin to undergo a Powerstroke (myotrop effect ([1-6].

The aim of our study is to determine the beta-tubulin level, which is one of the cardiomyocyte structure proteins in serum and to investigate the relationship with heart failure, and also to investigate the importance of microtubulins and microtubule inhibition via β1 -tubulin levels, which are the building blocks of microtubulins of cytoskeleton filaments in stage 4 heart failure with reduced ejection fraction.

METHODS

The study of " Evaluation of the importance of microtubules and microtubule inhibition through blood β1- tubulin levels in patients with heart failure with reduced ejection fraction "by Istanbul University Cerrahpaşa, Ethics Committee of Cardiology Institute dated 12.12.2018 and B 08.06 YÖK 2.İ.Ü.E.50.0.05.00 / 12 No. After approval of the study protocol by the institutional ethics committee, informed written consent was obtained from all the subjects. The study was performed in compliance with the ethical standards laid down in the 1975 Declaration of Helsinki.

The sample size was calculated by the formula: n=Z121 α/2S2÷d2 (n=80=1.962×(1-0.55/2)×0.502÷0.052)(N= Population Size; n=Sample Size; S= Standard Error;0.05 (5%) (Confidence Interval [CI] 95%); Confidence Interval: upper=95%; Lower: 5%; The Z-values for confidence levels were: 1.645=90 percent confidence level; 1.96= 95 percent confidence level; 2.576 = 99 percent confidence level (Z for p=0.05, 0.01, 0.001 are1.96, 2.58 and 3.28 Z values respectively); d= RelativeStandard Error) and it was confirmed by automatic sample size calculator [14].

Design and definitions

Our study was designed as a prospective cross-sectional observational. Based on ESC 2016 heart failure guidelines, symptoms and findings, biochemical and echocardiographic parameters, and etiology of the disease were evaluated. A total of 80 patients with 50 HFrEF (25 with ischemic etiology and 25 with non-ischemic etiology), and 30 healthy subjects were included in the study. Half of the HFrEF patients included in the study were selected from ischemic dilated cardiomyopathy patients with ischemic etiology, while the other half were selected from non-ischemic dilated cardiomyopathy patients without ischemic etiology [15]. For the control group, healthy individuals without heart failure with similar demographic characteristics were included in the study. In addition, low-dose colchicine (0.5-1 mg), was added to the treatment for at least three months in our 13 patients HFrEF with non-ischemic etiology for various reasons such as pericardial effusion accompanying HFrEF, and/or who developed hyperuricemia as a result of intensive diuretic therapy.

Exclusion criteria

Patients with the following characteristics were excluded from the study: under 18 old, being older than 80, having a history of hospitalization due to acute coronary syndrome in the last 30 days, end-stage renal disease patients, To have an active infection, having a life expectancy of less than 1 year (such as malignancies), due to noncardiac reasons, to have advanced valve disease.

Study population characteristics

The demographic characteristics of all patients included in the study and all individuals included in the control group; Age, gender was recorded in appropriate forms in the study forms, and symptom status was classified according to the NYHA symptom evaluation system. Comorbid diseases; hypertension, Diabetes Mellitus, hyperlipidemia, ischemic heart disease history was recorded in the study form. Heart rhythms (sinus rhythm / atrial fibrillation/battery rhythm), presence of the device (Pacemaker/ ICD/CRT-D), and habits of the patients and control group; cigarettes, alcohol, and medications they used were questioned and written to the appropriate places in the study forms. In the study group and control group, the diagnosis of DM was based on at least one history of antidiabetic drug use or HbA1c level> 6.5%.

Recent biochemical tests of all patients and control subjects included total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, glucose, HbA1c, creatine, eGFR, total protein, albumin, ALT, AST, NT-ProBNP levels and the hemogram parameters (hemoglobin, leukocyte, neutrophil, monocyte,

lymphocyte, basophil, platelet, MPV) were written in the appropriate places. No additional tests were performed to determine the necessary blood results of the patients. Glomerular filtration rates (GFR), of the patients were calculated using the MDRD formula. Also, echocardiography parameters of all patients and control groups were examined in detail. EF, left atrium (LA), left ventricular end-diastolic diameter (LVD), right ventricular diameter (RVD), septum thickness (IVS), segmenter or global where the study forms were recorded in the appropriate places (Table 1-3). Coronary angiography and cardiac MSCT, MRI criteria were used for the differential diagnosis of ischemic and non-ischemic dilated cardiomyopathy.

Plasma β1 -tubulin levels determination

Consent form was obtained from the patients and control group, and blood samples were taken into 8 ml K-EDTA tubes with Monovette brand vacutainer at the time of application and centrifuged at 3000 rpm for 10 minutes. Serum samples obtained were stored at -80°C for further study. Plasma beta tubulin levels were determined using Human Tubulin Beta-1 Chain ELISA Kit (Bioassay Technology Laboratory. Each well was previously prepared according to the kit package insert. All reagents, standard solutions, and samples were prepared as instructed.

All reagents were be brought to room temperature before use. The 120µl of the standard (1280ng/L), with 120µl of standard diluent to generate a 640ng/L standard stock solution was reconstituted. The standard to sit for 15 mins with gentle agitation prior to making dilutions was allowed. Standard points by serially diluting the standard stock solution (640ng/L) 1:2 with standard diluent to produce 320ng/L, 160ng/L, 80ng/L, and 40ng/L solutions were duplicated. Standard diluent serves as the zero standards (0 ng/L). Any remaining solution was to be frozen at -20°C and used within one month.

Reagent content has consisted of those:

- Standard Solution
- Coated ELISA plate
- Standard solvent
- Streptavidin HRP
- A substrate solution
- B substrate solution
- Washing buffer
- Biotinylated Human TUBB antibody
- Sealing plate

Used materials:

- 37ºC ± 0.5ºC incubator
- Absorbent paper
- Adjustable single channel pipettes
- Clean tube
- Distilled water
- Microwell strip reader (reading at 450 nm)

Reagent Preparation and plasma β1 -tubulin levels determination

Washing Buffer (1x wash buffer)

1. 50 ml of 20x concentrated wash buffer was transfered to 1.1000 ml graduated mesure. It was brought to 1000 ml with distilled or deionized water in the glass.

2. Avoiding the formation of foam was mix gently.

3. It was Transfered to a clean bottle and store at 2-25 ° C (1x Wash buffer remains stable for 30 days).

Serum samples taken and stored at -80 ° C were allowed to dissolve at room temperature in the laboratory before measurements were taken. It was diluted before the test. If crystals were formed in the concentrated buffers, they were completely dissolved by gentle heating.

All reagents, standard solutions, and samples were prepared. All reagents, solutions, and samples were brought to room temperature before use. The number of strips planned to use was determined and separated. The concentrated Biotin-Conjugate solution was diluted 1: 100 with Assay Buffer (1x), in a clean plastic tube.

The concentrated Streptavidin-HRP solution was diluted 1: 100 in a clean plastic tube with Assay Buffer (1x). Then the reagent, diluted serum sample mixture, Biotin-Conjugate solution, Streptavidin-HRP solution mixture were placed on the sealer plate and incubated at 37ºC for 60 minutes. The plate was then removed and the plate was washed 5 times with wash buffer. Solutions A and B were added and the mixture was allowed to stand for 10 min at 37ºC in the dark. Subsequently, Stop Solution was added to the mixture until the blue color turned yellow. The optical density value was then determined within 10 minutes using the Microwell strip reader (reading at 450 nm). Serum Beta Tubulin concentrations were plotted on the horizontal axis and the optical density on the vertical axis was plotted and serum Beta Tubulin concentrations were determined from this graph.

Statistical analysıs

Statistical analysis was performed by using SPSS (Statistical Package for Social Science), for Windows 23. 0 program. The student's t-test was used when the parameters providing normal distribution conditions were compared according to two independent groups. In the comparison of categorical values, independence control was performed by using Chi-Square ($\chi 2$) analysis. β1- tubulin levels of healthy individuals, ischemic dilated cardiomyopathy and non-ischemic dilated cardiomyopathy patients were compared with the Mann-Whitney U test. Statistical significance was accepted as $p \leq 0.05$.

RESULTS

The patient group consisted of 50 patients with low ejection fraction heart failure and a control group of 30 healthy volunteers with similar demographic characteristics. Twenty-five patients with heart failure were selected from patients with ischemic etiology and 25 patients with non-ischemic etiology. In addition, low-dose colchicine (0.5-1 mg), was added to the treatment for at

least three months in 13 patients with HFrEF with nonischemic etiology due to pericardial effusion accompanying HFrEF, and/or who developed hyperuricemia as a result of intensive diuretic therapy (Table 1-3) (Figure 1).

In our study,although serum β1-Tubulin levels of the all HFrEF patient group compared to the control groups did not reach statistical significance, increased serum β1-Tubulin 1levels were found in patients with HFrEF (p: 0.6) (Table 3A).

Table 1: Baseline demographic characteristics, biochemical properties, and echocardiographic parameters of the patients (N: 50) and control (N: 30) who participated in the study and drugs used by total Heart Failure reduced Ejection Fraction (HFrEF) Group.

Parameters	Patient (N: 50)	Control (N: 30)	P-value *
Age	59.1 ± 13.0	60.0 ± 8.7	0.74
Gender (male %)	43 (%86.0)	16(53.3%)	**0.001**
Diabetes Mellitus (%)	22(%44)	20(40%)	0.72
Hypertension (%)	29(%58)	22(%73,3)	0.16
Hyperlipidemia (%)	25(%50)	21(70%)	0.08
Cigaret (%)	24(%48)	16(53,3%)	0.64
Alcohol (%)	3(%6)	0	0.17
Rhythm (sinus rhythm)	38(76%)	27(90%)	0.12
Creatinine (mg / dl)	1.0 ± 0.3	0.8 ± 0.2	**0.002**
eGFR (ml / min)	75.5 ± 24.9	84.8 ± 18.7	0.05
Total cholesterol (mg / dl)	179.7 ± 47.8	200.5 ± 50.8	0.07
LDL cholesterol (mg / dl)	122.6 ± 42.8	134.3 ± 48.1	0.27
HDL cholesterol (mg / dl)	42.0 ± 13.2	50.9 ± 11.5	**0.004**
Triglyceride (mg / dl)	168.5 ± 140.0	149.5 ± 66.2	0.48
ALT (U/L)	39.7 ± 71,4	21.9 ± 9,4	0.18
AST (U/L)	24.3 ± 30.2	21.1 ± 6.6	0.26
Total protein (mg / dl)	6.7 ± 0.7	7.2 ± 2.0	0.07
Albumin (mg / dl)	3.8 ± 0.4	4.3 ± 0.2	**0.003**
Glucose (mg / dl)	132.1 ± 56.2	115.8 ± 27.0	0.14
HbA1c (%)	6.6 ± 1,2	6.5 ± 1.1	0.84
Hemoglobin (g / dl)	13.4 ± 1.5	14.1 ± 1.3	**0.05**
Leukocytes (mm^3)	7800 ± 2400	7400 ± 2600	0.54
Lymphocyte (mm^3)	1900 ± 1000	2100 ± 700	0.21
Neutrophil (mm^3)	5100 ± 1400	4600 ± 1700	0.16
Platelets (x1000)	220 ± 81	244 ± 59.6	0.16
MPV	9.0 ± 1.0	11.3 ± 1.2	0.18
LVD (mm)	62.1 ± 9.4	48.4 ± 4.2	0.000
LA (mm)	46.7 ± 7.7	37.5 ± 4.5	0.000
RVD (mm)	25.4 ± 2.9	23.9 ± 2.3	**0.052**
IVS (mm)	9.7 ± 1.7	10.7 ± 1.0	**0.014**
TAPSE (mm)	19.5 ± 2.8	22.4 ± 2.3	0.000
Drugs	Usage		
ACEI/ARB	49 (98) %)		
Beta Blocker	48 (96%)		
Mineralocorticoid antagonists (MRA)	31(62%)		
furosemide	39 (78%)		
Ivadbradine	3 (6%)		
Digoxin	15 (30%)		
Colchicine	13 (26%)		

*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.

Table 2: Demographic, biochemical properties, treatment differences, and echocardiographic parameters of the patients with ischemic (N: 25) and non-ischemic (N: 25) etiology of heart failure patients included in the study (mean ± SD).

Parameters	Ischemic (N: 25)	Non-ischemic (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%)	0.001
Cigaret (%)	18 (72%)	6 (24%)	0.001
Alcohol (%)	1 (4%)	2 (8%)	0.5
Rhythm (sinus rhythm)	20 (80%)	18 (72%)	0.5
Creatinine (mg / dl)	1.1 ± 0.3	1.0 ± 0.3	0.20
eGFR (ml / min)	69.5 ± 21.7	81.5 ± 26.7	0.88
Total cholesterol (mg / dl)	175.3 ± 50,9	184.3 ± 45	0.52
LDL cholesterol (mg / dl)	123.5 ± 48,7	121.7 ± 36,8	0.88
HDL cholesterol (mg/ dl)	42.9 ± 12.1	41.0 ± 14.5	0.65
Trigliserid (mg/dl)	141.0 ± 83,0	197.3 ± 179,1	0.17
ALT (U/L)	22.7 ± 11,4	57.3 ± 99.3	0.09
AST (U/L)	18.7 ± 7.4	36.4 ± 41.0	0.04
Total protein (mg / dl)	6.95 ± 0,8	6.55 ± 0.6	0.15
Albumin (mg / dl)	3.8 ± 0.5	3.9 ± 0.4	0.64
Glucose (mg / dl)	138.7 ± 48.4	125.3 ± 63,7	0.41
HbA1c (%)	6.9 ± 1.1	6.3 ± 1.2	0.10
Hemoglobin (g / dl)	12.9 ± 1.4	13.9 ± 1.5	0.03
Leukocytes (mm^3)	8000 ± 2300	7600 ± 2500	0.51
Lymphocyte (mm^3)	1700 ± 850	2200 ± 1100	0.11
Neutrophil (mm^3)	5500 ± 1500	5000 ± 1300	0.08
Platelets (x1000)	220 ± 74	220 ± 90	0.91
MPV	9.1 ± 1.0	8.9 ± 1.0	0.37
ACEI / ARB	24 (%96)	25 (100%)	0.31
Beta Blocker	24 (%96)	24 (96%)	1
MRA	16 (%66.7)	15 (62.5%)	0.76
Furosemide	18 (%72)	21 (84%)	0.3
Ivabradine	2 (%8)	1 (4%)	0.55
Digoxin	3 (%12)	12 (48%)	0.005
Colchicine	-	13(52%)	0.0001
EF (%)	33.0 ± 6.8	29.6 ± 5.0	0.05
LVD (mm)	58.1 ± 8.2	66.1 ± 9.0	0.002
LA (mm)	45.6 ± 6.1	47.7 ± 9.0	0.35
RVD (mm)	25.66 ± 2.9	25.2 ± 3.0	0.57
IVS (mm)	10.1 ± 1.8	9.4 ± 1.6	0.15
TAPSE (mm)	19.6 ± 2.9	19.4 ± 2.7	0.80

When the relationship between ejection fraction and serum β1-Tubulin levels of HFrEF patient group in the study was examined, we found that patients with lower ejection fraction had correlated higher β1-Tubulin levels. (p: 0.018, R^2: 0.086).

When HFrEF groups with the control group were compared, renal function (creatinine and eGFR), were better in the control group (Table 1) (p: 0.002.; P: 0.05 respectively).

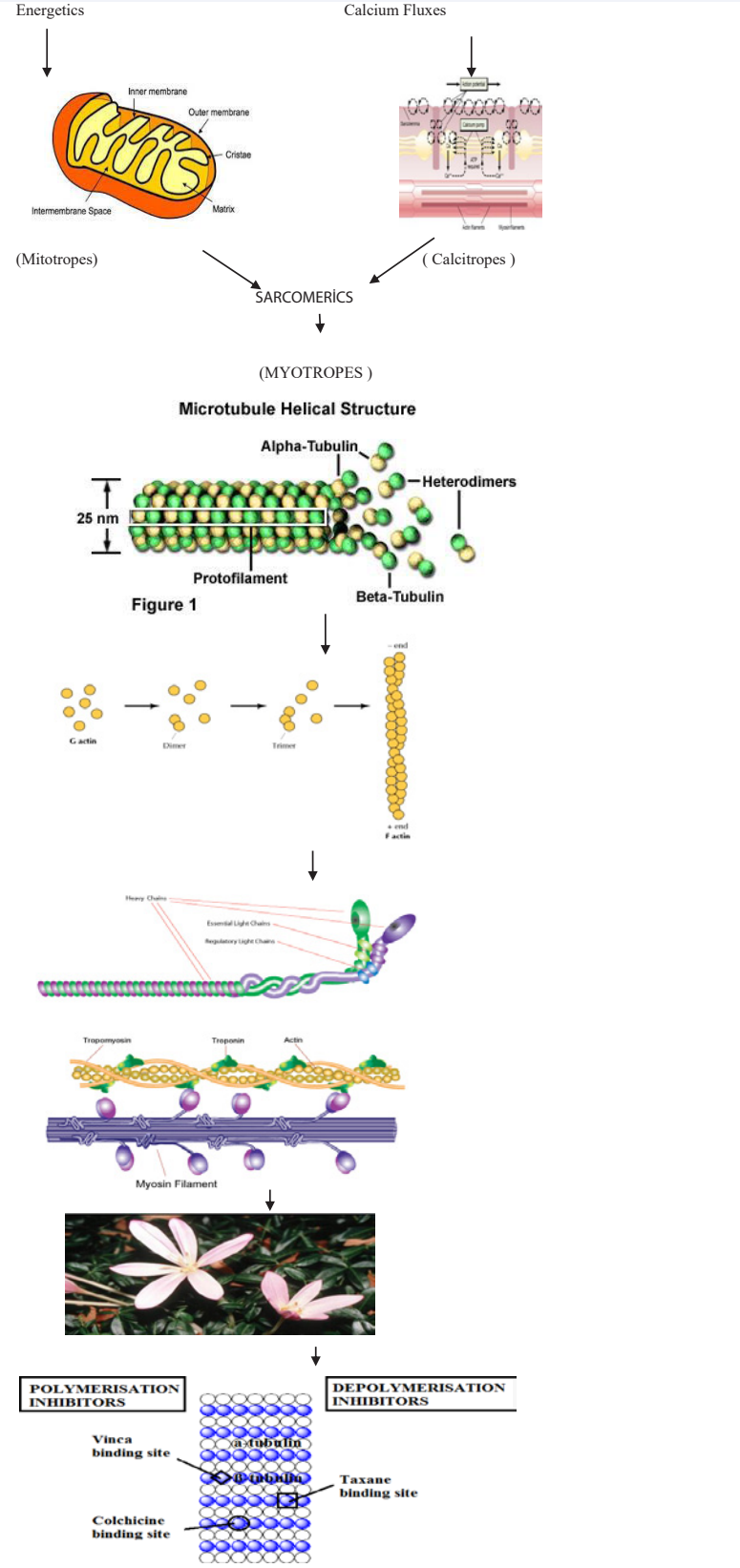

Figure 1 CENTRAL ILLUSTRATION: The Myocardial contractile apparatus and classes of therapeutic agents, and colchicine.
Dipnotes: Actin microfilaments are more effective in the microenvironment, and in case of inhibition of microtubules such as the use of colchicine, they may be more bound to myosin heads and increase the number of myosin heads able to bind to actin to undergo a Powerstroke (myotrope effect) such as omecamtiv mecarbil.

When HFrEF groups with ischemic and non-ischemic etiology were compared, renal functions were similar (p=0.88). There was also no significant correlation between creatinine and eGFR levels and Serum β1 –tubulin levels (p: 0.482) (Table 1,Table 2).

Also, in subgroup analysis, β1- tubulin levels had increased in ischemic HFrEF patients according to the non-ischemic subgroup (p: 0.26) (Table 2,Table 3 parameters B).

Besides, in the subgroup analysis of non-ischemic HFrEF patients used colchicine (n=13), was detected decreased the levels of β1- tubulin (p=0.29), and NT*proBNP (p=0.69). But, for at least three months low dose colchicine used patient subgroup had better EF (p=0.009), and smaller diastolic left ventricular diameters (p=0.002) respectively (Table 3 parameters C).

DISCUSSION

The goal of optimizing cardiac function, the load-independent contractile activity of the myocardium, is a valuable target for novel therapeutics to treat HFrEF. It remains unclear whether pharmaceuticals that use mechanisms other than Ca^{2+} to directly boost myocardial contractile action will demonstrate better efficacy and safety than currently available agents.The underlying Ca^{2+}-centric mechanism of these agents may be the dual-edged sword that causes both their inotropic and detrimental effects. Three mechanisms include 3 basic myocardial processes: Ca^{2+}-based regulation; the molecular motor and sarcomeric scaffolding; and energetics. Agents that primarily alter Ca^{2+} intracellular concentrations should be called cardiac calcitropes, those that directly affect myosin or other components of the sarcomere should be called cardiac myotropes, and those that alter myocardial energetics should be called cardiac mitotropes([1,2].

In our study, although serum β1-Tubulin levels of the all HFrEF patient group compared to the control groups did not reach statistical significance, increased serum β1-Tubulin level was found in patients with HFrEF (97.4 ± 174.8 vs. 81.3 ± 172.7 p> 0.05) (p: 0.6) (Table 3A).When the relationship between ejection fraction and serum β1-Tubulin levels of HFrEF patient group in the study was examined, we found that patients with lower ejection fraction had correlated higher β1-Tubulin levels. (p: 0.018, R^2: 0.086). Also, in subgroup analysis, β1- tubulin levels had increased in ischemic HFrEF patients according to the non-ischemic subgroup (108 ± 222 vs. 53 ± 95 p> 0.05) (p: 0.26) (Table 3 parameters B). Although we do not reach statistical significance, we think that this difference may be due to increased hemodynamic cardiac stress and increased fibrosis with ischemia similar to the mechanism explaining that NT-proBNP levels are high in ischemic heart failure. There are many molecular studies, biopsy, and autopsy and animal experiments to determine the relationship between cardiovascular diseases and β1- tubulin. β1- tubulin levels were increased in all these studies [16-18]. We could not find any study in the literature based on the determination of the plasma β1- tubulin levels that being one of microtubule activity markers in serum in patients with heart failure. In our study, although it does not address patients with acute heart failure, higher NT-proBNP levels found in the ischemic heart failure subgroup to the non-ischemic subgroup (2647 ± 2182 vs.1813 ± 1924 p <0.05). Our data were similar to those in the literature [19].

When HFrEF groups with ischemic and non-ischemic etiology were compared, renal functions were similar (p=0.88). There was also no significant correlation between creatinine and eGFR levels and Serum β1 –tubulin levels (p: 0.482) (Table 1,Table 3 parameters A).

Furthermore, in our study, higher creatinine and decreased eGFR levels were found in the heart failure group with low EF compared to the control group(p:0002;0,05) (Table 1). When HFrEF groups with ischemic and non-ischemic etiology were compared, renal functions were similar (Table 2). There was

Table 3: parameters*A,B,C. 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).

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

*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.

also no significant correlation between creatinine, eGFR values, and β1- tubulin levels, a microtubule dynamicity marker in mammals in the patient groups (p: 0.482). This proves that other mechanisms such as dehydration, electrolyte imbalance, and hypotensive condition are responsible for the deterioration of renal function in patients with HFrEF rather than microtubule activity [20].

Besides, in the subgroup analysis of non-ischemic HFrEF patients used colchicine (n=13), was detected decreased the levels of β1- tubulin ((37 ± 81 versus 97 ± 194 p> 0.05) (p=0.29), and NT*proBNP (2029.9 ± 2344 versus 2300 ± 2000 p> 0.05) (p=0.69), according to those who do not use colchicine. But, for at least three months low dose colchicine used patient subgroup had better EF((27.5 ± 3.2 vs 32.6 ± 6.4) p=0.009), and smaller diastolic left ventricular diameters (and 59.7 ± 8.9 vs 69 ± 7.6 (p=0.002), respectively over past at least three months after using colchicine (Table 3 parameters C). Our study gives the impression that the adverse remodeling of the left ventricle can be reversed by low dose colchicine.

Colchicine is a medication with a relatively low therapeutic index. Because high doses, especially in muscle cells can lead to vacuolization and myopathy should be avoided. Some recent studies have shown that colchicine may have positive effects on heart disease. Colchicine inhibits tubulin polymerization and myocyte apoptosis [10,12]. Colchicine accelerates misfolded protein are physiologically cleared from the cells by the protein quality control (PQC), system. PQC system controls dynamic selective autophagy [10,11]. Colchicine was reported to reduce NLRP3 Inflammasome and inflammatory cytokine storm (especially IL-1β and IL-18) and infarction size in MI [12]. Therefore, the sustained benefit of short-term colchicine treatment on survival, cardiac function, and ventricular remodeling after Mİ is to be beneficial [12]. Whereas in previous studies, the colchicine treatment of Heart Failure with Reduced Ejection Fraction (HrEF), has been reported to be conflicting [21,22].

Microtubules are cytoskeletal fibers, composed of αβ-tubulin heterodimers, which play a major role in mitosis, maintenance of cell shape and structure, and cell motility. Their formation is highly dynamic involving polymerization/depolymerization of the αβ-tubulin heterodimers, a process that is crucial for cell survival ([23]. Compounds that alter this equilibrium of assembly/disassembly between heterodimers induce, among other events, cell cycle arrest and apoptosis [23,24]. 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. Critical concentration differs from the positive (CC+), and the negative end (CC−), and under normal physiological conditions, the critical concentration is lower at the positive end than the negative end. The cytosolic concentration of the monomer subunit between the CC+ and CC− ends is what is defined as treadmilling in which there is growth at the plus end, and shrinking on the minus end. 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 [25].

According to our opinion;actin microfilaments are more effective in the microenvironment, and in case of inhibition of microtubules such as the use of colchicine, they may be more bound to myosin heads and increase the number of myosin heads able to bind to actin to undergo a Powerstroke (myotropes effect) [1,2]. Omecamtiv mecarbil (OM) is a novel, selective cardiac myosin activator that improves cardiac function, ventricular volumes, and NT-proBNP in patients with heart failure (HF), with reduced ejection fraction. Also, colchicine possibly may affects myosin with a similar mechanism such as omecamtiv mecarbil [26].

LIMITATIONS

Limitations of the study were non-randomized prospective cross-sectional observational design, relatively small number of patients, and whether there was not investigated the correlation between serum beta tubulin -1 levels and beta tubulin-1 levels measured in cardiac biopsy materials.

CONCLUSIONS

Our study gives the impression that the adverse remodeling of the left ventricle can be reversed by low dose colchicine.Thus, actin microfilaments are more effective in the microenvironment, and due to the inhibition of microtubules, they bind more to myosin heads (myotropic effect).Therefore, microtubule inhibitor drugs such as colchicine should be investigated for long time treatment options in heart failure. Also, we concluded to understand the role of structural proteins cardiomyocyte, especially β-tubulin levels in heart failure diagnosis and follow up further studies are needed.

CLINICAL PERSPECTIVES

Actin microfilaments are more effective in the microenvironment, and in case of inhibition of microtubules such as the use of colchicine, they may be more bound to myosin heads and increase the number of myosin heads able to bind to actin to undergo a Powerstroke (myotropes effect). Low dose colchicine regulates autophagy, microtubules inhibition, and vesicle trafficking in HFrEF.

TRANSLATIONAL OUTLOOK

Omecamtiv mecarbil (OM), is a novel, selective cardiac myosin activator that improves cardiac function, ventricular volumes, and NT-proBNP in patients with heart failure (HF), with reduced ejection fraction. Also, colchicine possibly may affects myosin with a similar mechanism such as omecamtiv mecarbil (OM).

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AUTHOR CONTRIBUTION FORM

Type of Contribution Description Contributors Conception

Constructing an idea or hypothesis for research and/or manuscript DESIGN Planning methodology to reach the conclusion: Nazmi Gültekin

Supervision: Organising and supervising the course of the project or the article and taking the responsibility: Nazmi Gültekin

Fundings: Providing personnel, environmental and financial support and tools and instruments that are vital for the project: No

Materials: Biological materials, reagents and referred patients: Nazmi Gültekin and Urfan Jafarov

DATA COLLECTION AND Taking responsibility in execution of the /OR PROCESSING experiments, patient follow-up, data management and reporting: Nazmi Gültekin; Emine Küçükateş; Urfan Jafarov

ANALYSIS AND/OR Taking responsibility in logical interpretation INTERPRETATION and presentation of the results: Nazmi Gültekin; Urfan Jafarov; Emine Küçükateş

LITERATURE REVIEW Taking responsibility in this necessary function WRITER Taking responsibility in the construction of the whole or body of the manuscript Nazmi Gültekin; Emine Küçükateş

CRITICAL REVIEW reviewing the article before submission not only for spelling and grammar but also for its intellectual content Nazmi Gültekin

ETHICS

The study of "Evaluation of the importance of microtubules and microtubule inhibition through blood β1- tubulin levels in patients with heart failure with reduced ejection fraction" by Istanbul University Cerrahpaşa, Ethics Committee of Cardiology Institute dated 12.12.2018 and B 08.06 YÖK 2.İ.Ü.E.50.0.05.00 / 12 No. After approval of the study protocol by the institutional ethics committee, informed written consent was obtained from all the subjects. The study was performed in compliance with the ethical standards laid down in the 1975 Declaration of Helsinki.

Ethical Review Board named

Istanbul University- Cerrahpaşa University Ethics Committee (Zerrin Yiğit; Murat Ersanlı; Ayşem Kaya).

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