# THE FIRST EVIDENCE FOR A DEFICIENCY OF COENZYME $Q_{10}$ IN PATIENTS WITH EBSTEIN ANOMALY

## Prvý dôkaz deficiencie koenzýmu $Q_{10}$ u pacientov s Ebsteinovou anomáliou

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**Abstract**
**Background:** Coenzyme $Q_{10}$ ($CoQ_{10}$) plays a crucial role in adenosine triphosphate synthesis within the mitochondrial respiratory chain and acts as a potent antioxidant, mitigating reactive oxygen species. Its deficiency has been documented in several cardiac disorders. However, coenzyme $Q_{10}$ levels in patients with Ebstein's anomaly (EA) remain unexplored.
**Methods:** This cross-sectional study evaluated endogenous $CoQ_{10}$ levels, as well as concentrations of α-tocopherol (αT), γ-tocopherol (γT), β-carotene, and lipid peroxidation products in plasma (PL) and thrombocytes (PLT), in both EA patients and a control healthy group. Cardiac magnetic resonance imaging (CMR) was performed exclusively in the EA group. Antioxidant levels were compared between EA patients and healthy controls, while within the EA group, additional comparisons were made based on indexed CMR-derived right ventricular (RV) end-diastolic volume (RVEDVi).
**Results**: This study involved 14 adult patients with confirmed EA and 18 healthy controls. The EA group had a mean age of 56 years, a mean BMI of 24.2 kg/m$^2$, and the following NYHA classifications: 35.7%/50%/14.3%/0% (I/II/III/IV). CMR confirmed the EA diagnosis with septal leaflet displacement of 16,1±6,73mm/m$_2$. $CoQ_{10}$ and γT levels in platelets were significantly lower in the EA group (81.50±26.70 vs 58.20±28.00pmol/10$^{-9}$ $^{PLT}$, P=0.02 and 157.00±169.00 vs 62.0±57.50pmol/10$^{-9}$ $^{PLT}$, P=0.01), while αT was higher (4980.00±3214.00 vs 7772.00±3202.00pmol/10$^{-9}$ $^{PLT}$, P=0.027). $CoQ_{10}$ in PL and PLT showed moderate positive correlations with CMR-derived RVEDVi (R=0.464, P=0.045 and R=0.476, P=0.047), while αT in plasma correlated negatively (R=-0.460, P=0.049). Subgroup analysis showed significantly lower $CoQ_{10}$ levels in both PL and PLT in the non-dilated RV group compared to the dilated RV (0.32±0.11 vs 0.50±1.19μmol/L$^{PL}$, P=0.046 and 43.50±13.77 vs 72.80±31.73pmol/10$^{-9}$ $^{PLT}$, P=0.044), with no other antioxidant differences between groups.
**Conclusion:** This study identified significantly reduced levels of $CoQ_{10}$, γT and increased αT in patients with EA compared to healthy controls. RVEDVi showed positive correlation with $CoQ_{10}$ and negative correlation with αT. The lowest $CoQ_{10}$

**Abstrakt**
**Úvod:** Koenzým Q10 ($CoQ_{10}$) zohráva kľúčovú úlohu pri syntéze adenozíntrifosfátu v dýchacom reťazci mitochondrií a súčasne pôsobí ako silný antioxidant. Jeho znížené hladiny boli potvrdené pri niektorých kardiovaskulárnych ochoreniach. U pacientov s Ebsteinovou anomáliou (EA) jeho koncetrácia doteraz nebola skúmaná.
**Metódy**: Realizovaná bola prierezová štúdia hodnotiaca koncentráciu $CoQ_{10}$, α-tokoferolu (αT), γ-tokoferolu (γT), β-karoténu a produktov peroxidácie lipidov u pacientov s EA a zdravej kontrolnej skupiny v trombocytoch (PLT) a v plazme (PL). U pacientov s EA bola realizovaná magnetická rezonancia srdca (CMR). Hladiny antioxidantov boli porovnané medzi pacientmi s EA a kontrolnou skupinou. V skupine EA bola vykonaná podskupinová analýza na základe indexovaného objemu pravého komory (RV) v diastole (RVEDVi) podľa CMR.
**Výsledky**: Zaradených bolo 14 dospelých pacientov s EA a 18 zdravých kontrol. Priemerný vek v skupine EA bol 56 rokov, BMI 24,2 kg/m$^2$ a zastúpenie NYHA: 35,7/50/14,3/0 % (I/II/III/IV). CMR potvrdila diagnózu EA s priemerným posunom septálneho cípu o 16,1±6,73 mm/m$_2$. Koncentrácia $CoQ_{10}$ a γT v PLT boli signifikantne nižšie v skupine EA v porovnaní s kontrolnou skupinou (81,50 ± 26,70 vs 58,20 ± 28,00 pmol/10$^{-9}$, P = 0,02 a 157,00 ± 169,00 vs 62,0 ± 57,50 pmol/10$^{-9}$ $^{PLT}$, P = 0,01), koncentrácie αT boli vyššie (4980,00 ± 3214,00 vs 7772,00 ± 3202,00 pmol/10$^{-9}$ $^{PLT}$, P = 0,027). Koncentrácia $CoQ_{10}$ v PL aj PLT pozitívne korelovala s RVEDVi (R = 0,464, P = 0,045 a R = 0,476, P = 0,047), koncentrácia αT v PL korelovala negatívne (R = -0,460, P = 0,049). Analýza podskupín potvrdila signifikantne nižšie hladiny $CoQ_{10}$ v PL aj PLT u pacientov s nedilatovanou RV v porovní s pacientmi s dilatovanou RV (0,32 ± 0,11 vs 0,50 ± 1,19 μmol/L $^{PL}$, P = 0,046 a 43,50 ± 13,77 vs 72,80 ± 31,73 pmol/10$^{-9}$ $^{PLT}$, P = 0,044), kocentrácie iných antioxidantov neboli významne odlišné medzi skupinami.
**Záver**: Uvedená štúdia potvrdila signifikantne znížené hladiny $CoQ_{10}$, γT a zvýšené hladiny αT u pacientov s EA v porovnaní so zdravými kontrolami. Objem RV pozitívne koreloval s $CoQ_{10}$ a nagatívne s αT. U pacientov s nedilatovanou RV boli potvrdené najnižšie hladiny $CoQ_{10}$. Koncentrácie antioxidantov môžu súvisieť s progresiou EA, pričom deficit $CoQ_{10}$ môže odrážať

levels were observed in patients with EA and non-dilated RV. Antioxidants may be linked to EA disease progression, while $CoQ_{10}$ deficiency may reflect intrinsic myocardial abnormalities (Tab. 5, Fig. 3, Ref. 26). *Text in PDF www.lekarsky.herba.sk.*
*KEY WORDS:* coenzyme $Q_{10}$, Ebstein anomaly, antioxidants, cardiomyopathy.
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primárne ochorenie myokardu RV (Tab. 5, Fig. 3, Ref. 26). *Text v* PDF www.lekarsky.herba.sk.
*KĽÚČOVÉ SLOVÁ:* koenzým $Q_{10}$, Ebsteinova anomália, antioxidanty, kardiomyopatia.

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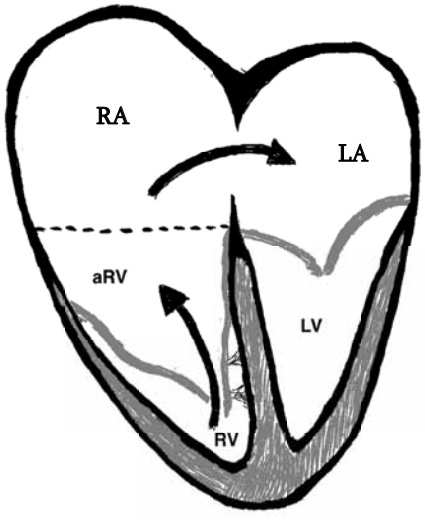
## Introduction

Ebstein's anomaly (EA), a rare example of a non-shunt congenital heart disease (CHD), is characterized by both morphological and functional abnormalities of the tricuspid valve (TV). It arises from incomplete delamination of the valve leaflets from the myocardium during embryonic development, resulting in the pathognomonic apical displacement of the septal leaflet of the TV (>8 mm/m$^2$). This condition leads to typical structural and functional changes, including the formation of the so-called atrialized portion of the right ventricle (aRV), a reduction in the functional right ventricular (RV) volume, and significant volume overload due to severe tricuspid regurgitation (TR) (1). A schematic representation of EA is shown in Figure 1. As with most CHDs, cardiac magnetic resonance imaging (CMR) serves as the gold-standard diagnostic method, providing detailed information about RV volume and function. These parameters are critical for determining the patient's prognosis and guiding further management (3). Currently, data on endogenous levels of coenzyme $Q_{10}$ ($CoQ_{10}$), antioxidants, and oxidative stress in patients with EA are lacking.

**Picture 1. Schematic representation of Ebstein's anomaly.**




aRV - atrialised right ventricle, LA - left atrium, LV - left ventricle, RA - right atrium, RV - right ventricle
Modified from 1.

$CoQ_{10}$ is a naturally occurring molecule that plays a fundamental role in energy production. Among other locations, it is found in the mitochondria, where it facilitates energy production in the form of adenosine triphosphate (ATP) through the process of oxidative phosphorylation. As a mobile molecule, it facilitates the transfer of electrons from Complex I and Complex II to Complex III of the mitochondrial respiratory chain and simultaneously, it generates a proton gradient necessary for the activity of ATP-synthase (Complex V). A byproduct of oxidative phosphorylation is the generation of reactive oxygen species (ROS). Extra-mitochondrial functions of $CoQ_{10}$ are largely represented by its strong antioxidant properties that protects against cellular damage from free radicals, including ROS (4). An imbalance between the concentration of antioxidants (e.g. $CoQ_{10}$, α-/γ-tocopherol, β-carotene) and ROS leads to oxidative stress, which damages tissues in several ways, including lipid peroxidation resulting in formation of thiobarbituric acid reactive substances (TBARS) (5).

Constant and sufficient ATP production is crucial for the contraction and relaxation of myocardium. Consequently, mitochondria account for up to 40% of cardiomyocyte volume and are the primary sources of both ATP and ROS (6). Therefore, maintaining an adequate concentration of $CoQ_{10}$ is vital for proper heart function, as it plays a crucial role in energy production and in mitigating oxidative stress.

In young and healthy individuals, $CoQ_{10}$ is produced in adequate amounts, ensuring both its main functions. However, with aging, the endogenous production of $CoQ_{10}$ declines, and its deficiency has also been noted in various pathophysiological conditions (7). In the 1970 Karl Folkers with colleagues the first time established reduced levels of $CoQ_{10}$ in blood and cardiac tissue in patients with congestive heart failure, with the degree of deficiency correlating with the severity of heart failure (8). Deficit of $CoQ_{10}$ in heart may lead to failure in mitochondrial bioenergetics and to reduction of antioxidant capacity of the myocardium (9).

Numerous subsequent studies have demonstrated low levels of $CoQ_{10}$ or mitochondrial dysfunction in various cardiovascular diseases, ranging from heart failure and different cardiomyopathies to pulmonary hypertension (10-11). Little attention, however, has been given to CHD, particularly in adulthood. Understanding their interrelationship could shed light on the patomechanism underlying RV dilation and dysfunction in EA.

The aim of this pilot study was to determine the endogenous levels of total $CoQ_{10}$ (ubiquinone + ubiquinol), α-tocopherol (αT), γ-tocopherol (γT), β-carotene, and parameters of lipid peroxitation TBARS in patients with EA, compare them to healthy population, and evaluate their relationship to the severity of RV dilation.

## Material and Methods

### *Subjects*

Adult patients (≥18 years) with EA, defined as septal leaflet displacement >8 mm/m$^2$), were selected from Slovak National Registry of Ebstein´s Anomaly (SNREA) and reviewed for inclusion. Exclusion criteria were: (i) previous cardiac surgery, (ii) concomitant congenital heart disease (except hemodynamically non-significant atrial septal defect), (iii) severe systemic disorders (e.g. chronic kidney disease defined as glomerular filtration rate < 60 mL/min, liver cirrhosis, cancer, obesity), (iv) uncontrolled cardiovascular disease (e.g. resistant arterial hypertension, non-sinus tachycardia), (v) statin intake, (vi) $CoQ_{10}$ and vitamin E supplementation, (vii) regular alcohol consumption, (viii) smoking, (ix) signs of acute infection

In a subgroup analysis the EA population was devied into two groups: EA with dilated RV (D-RV) and EA without RV dilatation (ND-RV). The classification was based on CMR-derived end-diastolic volume index of RV (RVEDVi) with a cutoff value of 105 mL/m$^2$, as published in previous literature (12).

Inclusion criteria for control group were: (i) absence of chronic medication, (ii) no coenzyme Q10 supplementation, and (iii) no vitamin E supplementation. Exclusion criteria: (i) systemic diseases, (ii) obesity, (iii) smoking, (iv) regular alcohol consumption and (v) signs of acute infection.

### *Cardiac magnetic resonance data*

CMR was performed the same day as laboratory analysis using a standard and commercialy available GE 3 Tesla scanner. The diagnosis of EA was confirmed by measuring the displacement of the septal leaflet from the plane of the mitral valve annulus to the origin of the septal cusp during ventricular systole. TV regurgitation severity (TR, %) was calculated from the difference between RV stroke volume (SV) and PA forward flow, expressed as a proportion of RV-SV. Volume of both - „true" RV and LV was quantified and indexed to BSA in end-diastole (RVEDVi, LVEDVi, mL/m$^2$) and end-systole (RVESVi, LVESVi, mL/m$^2$). Systolic function of both ventricles expressed as EF (%) was calculated. CMR postprocessing and analysis was completed by single experienced radiologist trained in congenital heart disease imaging. CMR was conducted only in EA patients group.

### *Laboratory parameters*

In platelets (PLT) from both groups, levels of γT, αT, and total $CoQ_{10}$ were measured. In plasma, concentrations of γT, αT, β-carotene, total $CoQ^{10}$ and TBARS were determined.

**1. Platelets isolation**

PLT were isolated from whole blood (13) as described previously (14) and counted on hematological analyzer Mindray BC-3600 (Mindray, China).

**2. Coenzyme $Q_{10\text{-TOTAL}}$, α-tocopherol, γ-tocopherol, and β-carotene determination in plasma**

Concentrations of $CoQ_{10}$ (ubiquinone + ubiquinol) and lipophilic vitamins in plasma were determined simultaneously by a modified HPLC method with spectrophotometric detection (15,16). For the oxidation of ubiquinol to ubiquinone, 100 µL of 1,4-benzoquinone (2 mg/1 mL double-distilled water) was added to a tube with 500 µl of blood or plasma and vortexed for 10 seconds (17). After 10 minutes of incubation at room temperature, 2 mL of a mixture of hexane/ethanol (5/2 v/v) was added. The tubes were shaken for 5 minutes and centrifuged at 1,000 g for 5 minutes. The hexane layer was separated and extraction procedure was repeated with 1 mL of the extraction mixture. Collected organic layers were evaporated under nitrogen at 50 °C. The residues were taken up in 99.9 % ethanol and injected into a reverse phase HPLC column. Elution was performed with methanol/acetonitrile/ethanol (6/2/2 v/v/v) at a flow rate of 0.9 mL/min. The concentrations of $CoQ_{10\text{-TOTAL}}$, αT, γT and β-carotene were detected with an UV-detector at 275 nm, 295 nm, and 450 nm, respectively, using external standards. Data were collected and processed with a CSW32 chromatographic station (DataApex Ltd). Concentrations of analyzed substances were calculated in µmol.l$^{-1}$.

**3. Coenzyme $Q_{10}$, α-tocopherol and γ-tocopherol determination in PLT**

Isolated human PLT (150–250 millions) were disintegrated with 500 µl of cold methanol (18). Oxidation of ubiquinol to ubiquinone was performed with 1,4-benzoquinone as described for plasma extraction. The cell suspension was extracted with 2 ml hexane by shaking for 5 minutes. After centrifugation, organic layer was separated, evaporated and measured as described above. Concentrations of analyzed substances were calculated in pmol.10$^{-9\ PLT}$.

**4. TBARS determination in plasma**

A parameter of oxidative stress TBARS, was determined by a spectrophotometric method (19).

The study was carried out according to the principles expressed in the Declaration of Helsinki, and the study protocol was approved by the Ethical Committee of Slovak Medical University No. 02/2024 from 11.3.2024. Written informed consent was obtained from each subject prior to enrollment in the study.

**Data analysis**

Data were electronically recorded, verified, and processed using Microsoft Office Excel, followed by statistical analysis conducted with JASP software (version 0.14.1, JASP Team 2020, https://jasp-stats.org). Continuous variables are expressed as means with standard deviations, and categorical variables are reported as absolute values. The normality of data distribution was assessed using the Shapiro-Wilk test. Comparisons of continuous variables were performed using the paired Student's t-test or the Wilcoxon signed-rank test, depending on data distribution. Categorical variables were compared using the Chi-square test or Fisher's

exact test. A P-value of less than 0.05 was considered indicative of statistical significance

## Results

### *Patients' characteristics*

In total, 14 patients with confirmed EA and listed in SREA were fit for inclusion: 12 women, 2 men with a median age of 56 ± 11.3 years and a mean body mass index (BMI) of 24.2 ± 6,69 kg/m$^{-2}$. NYHA functional classes were 35.7%, 50.0%, 14,3% for NYHA I, II and III, respectively. There was no patient in NYHA class IV.

The control group consisted of 18 healthy subjects (10 men and 8 women) without medical therapy, median age 50.4 ±12.2 years, median BMI of 25.9 ± 3.45 kg/m$^{-2}$. The basic physical and clinical characteristics of control group and EA group are summarized in Table 1.

**Table 1. Cohort characteristics - controls and patients.**

| Characteristics | Control (mean ± SD ) | Patients-EA (mean ± SD) |
|---|---|---|
| N, number | 18 | 14 |
| Age, years | 50.4±12.2 | 49,2±11.3 |
| Sex, female/male | 10/8 | 12/2 |
| BMI, kg/m$^2$ | 25.9±3.45 | 24.2±6.69 |
| **Medications** | | |
| Loop diuretics | 0 (0.0%) | 3 (21.4%) |
| ACEI/ARBs | 0 (0.0%) | 3 (21.4%) |
| B-blockers | 0 (0.0%) | 7 (50.0%) |
| Aldosteron blockers | 0 (0.0%) | 4 (28.6%) |
| Anticoagulants and antiplatelets | 0 (0.0%) | 6 (42.3%) |
| Dietary supplements | 0 (0.0%) | 0 (0.0%) |
| **NYHA** | | |
| I, n | na | 5 (35.7%) |
| II, n | na | 7 (50.0%) |
| III, n | na | 2 (14.3%) |
| IV, n | na | 0 (0.0%) |
| **Comborbidities** | | |
| Arterial hypertension | 0 (0.0%) | 4 (28.6%) |
| Diabetes mellitus | 0 (0.0%) | 1 (7.1%) |
| Arrhythmia | 0 (0.0%) | 9 (64.3%) |
| COPD/Bronchial asthma | 0 (0.0%) | 2 (14.3%) |
| Hepatopathy | 0 (0.0%) | 2 (14.3%) |
| Hyperlipidemia | 0 (0.0%) | 6 (42.3%) |
| Chronic kidney disease | 0 (0.0%) | 1 (7.1%) |
| Coronary artery disease | 0 (0.0%) | 1 (7.1%) |

ACEI - angiotensin-converting-enzyme inhibitors; ARBs - angiotensin receptor blockers; BMI - body mass index; COPD – chronic obstructive pulmonary disease; NYHA – New York Heart Association; SD – standard deviation

### *Cardiac magnetic resonance*

In EA group diagnosis was confirmed by CMR derived median septal leaflet displacement 13.1±6.73 mm/m$^2$. CMR parameters was performed only in EA group and are presented in Table 2.

**Table 2. Cardiac magnetic resonance imaging parameters.**

| CMR | EA group (mean ± SD) |
|---|---|
| Septal leaflet displacement, mm/m$^2$ | 16,1±6,73 |
| RV end-diastolic volume index, mL/m$^2$ | 113±37.50 |
| RV end-systolic volume index, mL/m$^2$ | 55.8±20.1 |
| RV ejection fraction, % | 51.5±7.94 |
| LV ejection fraction, % | 60±5.94 |
| Tricuspid regurgitation fraction, % | 46±18.6 |

CMR - cardiac magnetic resonance; EA - Ebstein's anomaly; LV - left ventricle; mm - milimeters; mL - mililiters; RV - right ventricle; SD – standard deviation

## Laboratory parameters

### *Group of all patients with Ebstein anomaly*

Concentration of endogenous $CoQ_{10}$ in PLT was significantly reduced compared to control group (81.50±26.70 pmol/10$^{-9PLT}$ vs 58.20±28.00 pmol/10$^{-9PLT}$, P=0.02). $CoQ_{10}$ in plasma was slightly reduced compared to control group, however this was not significant (0.46±0.12μmol/l vs 0.41±0.17μmol/l, P=0,16).

Concentration of γT in PLT was significantly reduced compared to the control group (157.00±169.00 pmol/10$^{-9\ PLT}$ vs 62.0±57.50 pmol/10$^{-9\ PLT}$, P=0.01). Concentration of αT PLT was singnificantly increased compared to control group (4980.00±3214.00pmol/10$^{-9\ PLT}$ vs 7772.00±3202.00pmol/10$^{-9\ PLT}$, P=0.027).

Concentration of αT in plasma was similar as in control group.

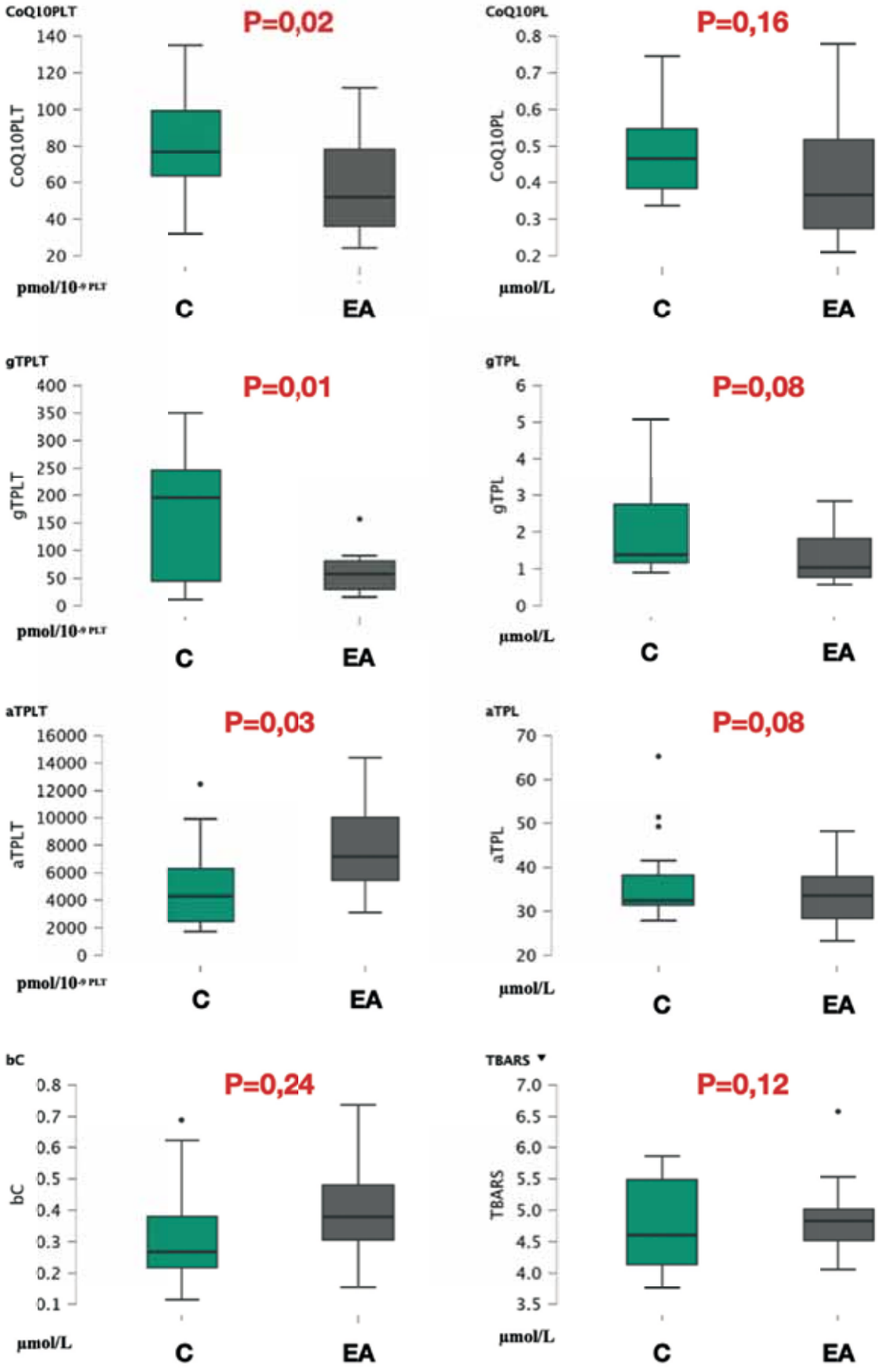
Concentration of γT, αT, β-carotene and TBARS in plasma were similar between both groups. Table 3 contains all antioxidants and TBARS levels in both groups, while Figure 2 provides their visual representation.

**Table 3. Antioxidants and TBARS levels in controls and EA group.**

| | Control (mean ± SD) | EA-all (mean ± SD) | P value |
|---|---|---|---|
| $CoQ_{10}$ PLT, pmol/10$^{-9\ PLT}$ | 81.50±26.70 | 58.20±28.00 | **0.02** |
| $CoQ_{10}$ PL, μmol/L | 0.46±0.12 | 0.41±0.17 | 0.16 |
| γ-tocopherol PLT, pmol/10$^{-9\ PLT}$ | 157.00±169.00 | 62.0±57.50 | **0.01** |
| γ-tocopherol PL, μmol/L | 2.09±1.42 | 1.34±0.71 | 0.08 |
| α-tocopherol PLT, pmol/10$^{-9\ PLT}$ | 4980.00±3214.00 | 7772.00±3202.00 | **0.03** |
| α-tocopherol PL, μmol/L | 36.40±9.80 | 33.80±7.12 | 0.41 |
| TBARS, μmol/L | 4.74±0.75 | 4.90±0.62 | 0.12 |
| β-caroten, μmol/L | 0.33±0.17 | 0.40±0.16 | 0.24 |

$CoQ_{10}$ - coenzyme Q10, EA - Ebstein's anomaly, PLT - platelets, PL - plasma, TBARS - thiobarbituric acid reactive substances, SD – standard deviation

**Figure 2. Comparison of antioxidant and TBARS levels between the EA and controls.**




aTPLT - α-tocopherol in platelets; aTPLZ - α-tocopherol in plasma; BC - β-carothene, CoQ10PLT - coenzyme $Q_{10}$ in platelets; C - control group, CoQ10PLZ - coenzyme $Q_{10}$ in plasma, EA - Ebstein's anomaly, gTPLT - γ-tocopherol in platelets, gTPLZ - γ-tocopherol in plasma, TBARS - thiobarbituric acid reactive substances

### *Correlations between imaging modalities and laboratory parameters*

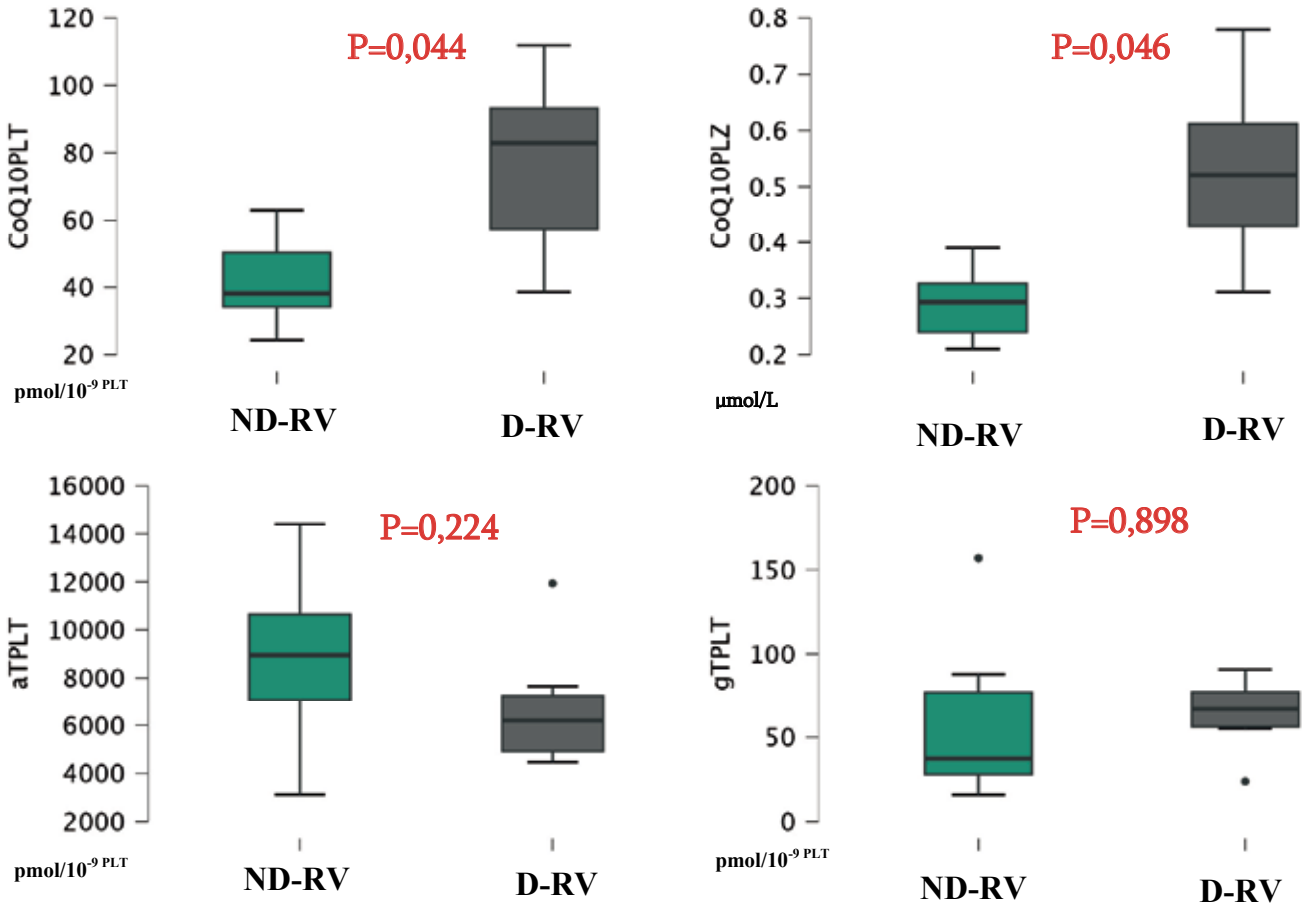
Correlation analysis between CMR-derived RV EDVi and antioxidant levels demonstrated a moderate positive correlation with high statistical significance for $CoQ_{10}$ levels in both plasma (R=0.471, P=0.045) and PLT (R=0.464, P=0.047). Among other antioxidants, a moderate negative correlation with high statistical significance was observed only for αT in PLT (R=-0,460, P=0,049). These findings are visually presented in Table 4 and Figure 3.

**Table 4. Correlation between RV EDVi and antioxindants / TBARS level.**

| | | | n | Pearson's r | p | Effect size (Fisher's z) | SE Effect size |
|---|---|---|---|---|---|---|---|
| EDVi | – | CoQ10PLT | 14 | 0.464 | 0.047 | 0.503 | 0.302 |
| EDVi | – | CoQ10PL | 14 | 0.471 | 0.045 | 0.511 | 0.302 |
| EDVi | – | BC | 14 | 0.082 | 0.390 | 0.082 | 0.302 |
| EDVi | – | TBARS | 14 | 0.028 | 0.463 | 0.028 | 0.302 |
| EDVi | – | gTPLT | 12 | −0.017 | 0.521 | −0.017 | 0.333 |
| EDVi | – | gTPL | 14 | 0.266 | 0.179 | 0.273 | 0.302 |
| EDVi | – | aTPLT | 14 | −0.460 | 0.049 | −0.497 | 0.302 |
| EDVi | – | aTPL | 14 | −0.164 | 0.287 | −0.166 | 0.302 |

aTPLT - α-tocopherol in platelets; aTPL - α-tocopherol in plasma; BC - β-carothene, CoQ10PLT - coenzyme $Q_{10}$ in platelets; CoQ10PL - coenzyme $Q_{10}$ in plasma, EDVi - end-diastolic volume index, gTPLT - γ-tocopherol in platelets, gTPL - γ-tocopherol in plasma, RV - right ventricle, SD - standard deviation, TBARS - thiobarbituric acid reactive substances

**Figure 3. Comparasion of selected antioxidants levels between ND-RV and D-RV.**




aTPLT - α-tocopherol in platelets, CoQ10PLT - coenzyme Q10 in platelets, CoQ10PLZ - coenzyme Q10 in plasma, D-RV - dilated right ventricle, gTPLT - γ-tocopherol in platelets, ND-RV - non-dilated right ventricle

### *Subgroup analysis*

In a subgroup analysis, comparison were made between forms of EA with D-RV (RVEDVi >105ml/m$^2$) and ND-RV (RVEDVi <105 ml/m$^2$) according to CMR. In ND-RV group concentration of $CoQ_{10}$ in both plasma and PLT wrere significantly reduced (0.32±0.11 μmol/l vs 0.50±1.19 μmol/l, P=0,046 and 43.50±13.77pmol/$10^{-9\ PLT}$ vs 72.80±31.73pmol/$10^{-9\ PLT}$ P=0,044 respectively) compared to D-RV. No significant difference between groups were shown in termes of γT, αT, β-carotene and TBARS. The comparison of both groups is presented in Table 5.

**Table 5 - Comparison of groups of EA patients with D-RV and ND-RV**

| | D-RV (mean ± SD) | ND-RV (mean ± SD) | P |
|---|---|---|---|
| **N (number)** | 7 | 7 | na |
| **Age (years)** | 55.50±3.73 | 43.90±13.00 | 0.061 |
| **Antioxidants** | | | |
| $CoQ_{10}$ PLT, pmol/$10^{-9\ PLT}$ | 72.80±31.73 | 43.50±13.77 | **0.044** |
| $CoQ_{10}$ PL, μmol/L | 0.50±1.19 | 0.32±0.11 | **0.046** |
| γ-tocopherol PLT, pmol/$10^{-9\ PLT}$ | 63.60±23.40 | 60.40±53.54 | 0.898 |
| γ-tocopherol PL, μmol/L | 1.57±0.87 | 1.11±0.48 | 0.242 |
| α-tocopherol PLT, pmol/$10^{-9\ PLT}$ | 6701.00±2588.49 | 8843.00±3582.68 | 0.224 |
| α-tocopherol PL, μmol/L | 33.30±5.48 | 34.40±8.96 | 0.778 |
| β-caroten, μmol/L | 0.36±0.15 | 0.44±0.17 | 0.343 |
| **TBARS, μmol/L** | 5.17±0.31 | 4.63±0.31 | 0.105 |
| **CMR parameters** | | | |
| LVEF, % | 57.20±6.97 | 60.30±4.99 | 0.368 |
| RVEF, % | 49.00±8.93 | 53.4±6.77 | 0.316 |
| RVEDVi, mm/m$^2$ | 142.00±31.44 | 83.70±9.45 | **<0.001** |
| RVESVi, mm/m$^2$ | 72.70±12.8 | 38.90±6.52 | **<0.001** |
| Indexed septal leaflet displacement, mm/m$^2$ | 17.60±8.07 | 14.70±5.28 | 0.438 |

$CoQ_{10}$ - coenzyme $Q_{10}$, CMR - cardiac magnetic resonance, D-RV - dilated right ventricl, EA - Ebstein's anomaly, LVEF - left ventricular ejection fraction, ND-RV - non-dilated right ventricle, PLT - platelets, PL - plasma, TBARS - thiobarbituric acid reactive substancese, RVEF - right ventricular ejection fraction, RVEDVi - right ventricular end-diastolic volume index, RVESVi - right ventricular end-systolic volume index, SD - standard deviation, TBARS - thiobarbituric acid reactive substances

## Discussion

To our knowledge this is the first study determining endogenous levels of $CoQ_{10}$ in platelets and in plasma of the patients with Ebstein´s anomaly (EA) as well as the concentrations of other antioxidants and lipid peroxidation products (TBARS).

The main findings are as follows:

1. $CoQ_{10}$ and γ-tocopherol (γT) are significantly reduced in patients with EA in both platelets and plasma compared to the healthy control group;
2. TBARS are slightly increased in the EA group compared to healthy individuals;
3. $CoQ_{10}$ levels exhibited a moderate positive correlation with high statistical significance with cardiac magnetic resonance-derived indexed right ventricular end-diastolic volume (CMR-RVEDVi);
4. α-tocopherol (αT) in platelets showed moderate negative correlation with high statistical significance with CMR-RVEDVi;
5. Patients with a dilated «true» RV (D-RV) have significantly lower levels of $CoQ_{10}$ compared to the patients with a non-dilated „true" RV.

EA is a rare congenital heart disease characterized by abnormal development of the tricuspid valve (TV). The degree of abnormal TV morphology and associated TV dysfunction varies among patients with EA. The typical consequence is primarily severe tricuspid regurgitation (TR) which leads to volume overload of the right ventricle (RV) and right-sided heart failure (HF) (1). Adaptation to volume overload involves a complex interplay of local and systemic changes. These mechanisms aim to maintain normal cardiac output and tissue perfusion; however, their prolonged activation can contribute to disease progression. Contra-regulatory mechanisms directly or indirectly influence mitochondrial functions. Since mitochondria are the primary sources of both adenosine triphosphate and ROS, their proper and balanced function is essential for a normal heart function.

Proposed mechanism of mitochondrial dysregluation in the context of HF is prolonged stimulation of angiotensin II (ATII), which leads to activation of NADPH oxidase family, located directly within mitochondria (20). Other example may be RV dilation, hallmark of RV adaptation to volume overload, which initially increases stroke volume according to LaPlace's law. Beyond a certain point, further dilation becomes maladaptive, increasing RV end-diatolic pressure. Animal studies confirmed, that mitochondrial dysfunction is associated with myocardial dilation, fibrosis, and cardiomyocyte dysfunction (21). The importance of RV volume and function is also reflected in the recommendations of the European Society of Cardiology for the management of EA patients and represents one of the indication criteria for TV repair/replacement (3).

Platelets are attractive sources of mitochondria that can be obtained less invasively, compared to tissue biopsy. Platelets are used for the assessment of organ-specific mitochondrial dysfunction that is relevant to clinical outcomes (17). Platelet mitochondrial dysfunction has been demonstrated in different studies on mitochondria-related diseases (22, 23). The high-resolution respirometry method allows the dynamic monitoring of mitochondrial function in human platelets. In this study, we used circulating platelets as well as plasma for the detection of changes of antioxidants in patients with EA.

In the present study, we observed significantly lower levels of $CoQ_{10}$ and γT, along with increased levels of αT in platelets from the EA group compared to the healthy population. One potential mechanism underlying the reduced endogenous levels of $CoQ_{10}$ could involve mutations in one or more COQ genes responsible for its biosynthesis (24). A member of the vitamin E family γT, functions as a fat-soluble antioxidant and a nitric oxide radical scavenger. Unlike αT, γT undergoes more rapid metabolism, primarily in the liver, via cytochrome P450 (CYP4F2) (25). A potential explanation for the decreased endogenous γT levels may be altered liver metabolism. Additionally, while TBARS levels were slightly elevated in the patient group, this difference was not statistically significant.

These findings suggest that maladaptive changes in EA patients may lead to impaired mitochondrial function, characterized by changes in antioxidant levels and increased oxidative stress, as reflected by slightly higher TBARS levels. This is consistent with the limited studies that have investigated antioxidant levels in other cardiovascular diseases. However, most of them have concentrated on left-sided heart disorders, with only a few specifically examining RV failure, primarily in the setting of pulmonary arterial hypertension (PAH) (11,21). Although certain parallels exist, the adaptive mechanisms differ between the left and right ventricles, as well as between pressure overload, which is typical of PAH, and volume overload, as seen in EA. Therefore, results from PAH studies cannot be extrapolated for EA.

Correlation analysis demonstrated a moderate and statistically significant positive association between RVEDVi and $CoQ_{10}$ levels in both platelets and plasma and negative moderate association with αT. No significant correlations were observed with other antioxidants or TBARS, as well as no correlation with RV systolic function. In a subgroup analysis we confirmed that patients with a ND-RV exhibited lower $CoQ_{10}$ levels compared to D-RV group, without differences between αT and γT.

It can be assumed that mitochondrial dysfunction and OS may serve as contributing factors to RV deterioration in EA. However, the relationship between EA and antioxidant concentrations is complex and nonlinear. Rather than absolute antioxidant concentrations, the relative balance of multiple antioxidants, not just a single one, in relation to ROS may be of greater significance. Based on aformentioned results we hypothesize that αT and possibly also γT are associated with the severity of the disease. However, different explanation is needed for the inverse relationship between $CoQ_{10}$ and RV volume, with a significant deficit observed in patients with ND-RV.

As previously published, EA patients with small RV demonstrate poorer performance in the 6-minute walk test and have higher NT-proBNP levels (12). Furthermore, EA is increasingly recognized as an RV cardiomyopathy rather than merely a valvular pathology. These intrinsic abnormalities of RV myocardium may result in the inability to adapt to volume overload with end-diastolic pressure and subsequent decrease in mitochondrial function (26). This reasoning is supported by the high prevalence of severe TR in the ND-RV group. Therefore, it is plausible that the $CoQ_{10}$ deficiency is linked to the primary pathology of cardiomyocytes. Further studies are needed to confirm this hypothesis, including longitudinal prospective measurements of antioxidant levels and mitochondrial function over time, ideally with invasively measured end-diastolic pressure in the right ventricle.

## Limitations

The low number of patients mainly in EA group is the main limitation of our study. Moreover, the majority of the patients are female, which further limits the generalizability of the study. Patients with severe systemic diseases (e.g., obesity, chronic kidney disease) were excluded, which may reduce the relevance of the results for more heterogeneous populations of patients with EA.

Another limitation is that the blood samples from EA group and control patient groups were collected at different time points. This may potentially introduce confounding factors to the interpretation of the results due to different epidemiological situation in different time periods. It is well known that various viruses (including COVID-19) affect mitochondrial metabolism as well as antioxidant levels. With the exception of signs of an acute infectious disease, an epidemiological history was not taken.

Furthermore, cardiac magnetic resonance imaging was performed only in patients with EA, so no comparison with a control group is available. This may limit the comprehensiveness of the assessment of relationships between imaging parameters and metabolic biomarkers.

## Conclusion

This study is the first to assess $CoQ_{10}$ levels, antioxidants, and lipid peroxidation products in patients with EA Our findings show significant changes in $CoQ_{10}$, γ-tocopherol, and α-tocopherol levels, with slightly increased lipid peroxidation products in EA patients compared to healthy controls. A positive correlation was found between $CoQ_{10}$ and right ventricular end-diastolic volume, while α-tocopherol showed a negative correlation. Patients with non-dilated right ventricle had lower $CoQ_{10}$ levels than those with dilated RV. These results suggest a complex relationship between mitochondrial dysfunction, oxidative stress, and RV adaptation to volume overload in EA. Further studies could to find out whether supplementation with $CoQ_{10}$ could be contribute to delaying the worsening of EA, or to stopping the progression of EA and to increasing the body's antioxidant capacity. Further studies are needed to confirm these findings.*

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**The authors declare** that the study was conducted in accordance with the ethical standards of the relevant committee responsible for clinical studies and the Helsinki Declaration of 1975, revised in 2000.

**Conflict of interest statement:** The authors declare that they have no conflict of interest

**Informed consent** was obtained from each patient prior to their inclusion in the study.

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