Original Paper

Estimation of Oxidative Stress Involvement by Superoxide Dismutase Variation in Cardiac Arrhythmias

Maria Cristina Bezná[1], Doina Cârstea[1],
Marinela Bezná[2], Cătalina Pisoschi[3], O. Istrătoaie[1],
D.O. Alexandru[4], C. Efrem[2], R.P. Melinte[5]

[1]Department of Cardiology, University of Medicine and Pharmacy of Craiova

[2]Department of Internal Medicine, University of Medicine and Pharmacy of Craiova

[3]Department of Biochemistry, University of Medicine and Pharmacy of Craiova

[4]Department of Medical Informatics, University of Medicine and Pharmacy of Craiova

[5]Department of Human Anatomy, University of Medicine and Pharmacy of Craiova

ABSTRACT: Cardiac arrhythmias, commonly diagnosed in young people, involve multiple etiopathogenic factors, including oxidative stress. Purpose: Evaluation of superoxide dismutase (SOD) variations as an antioxidant enzyme (with a physiological role in the dismutation of highly reactive oxygen free radicals into oxygen and water) in young patients with cardiac arrhythmias. Material and method: The study was conducted on a group of 40 young patients with a mean age of 34 years old, of both sexes, with non-lesional cardiac dysrhythmias, compared to a control group of 40 healthy subjects, determining for both groups the SOD serum level. Diagnosis of cardiac rhythm disorder was supported by electrocardiogram, imaging and laboratory investigations. Results: SOD recorded a 61% decrease of mean values in patients compared to controls. The decreasing variation was found in all arrhythmia types, as follows: atrial fibrillation (51,54%), sinus bradycardia (54,86%), atrial flutter (55,71%), extrasystolic ventricular arrhythmia (64,20%), extrasystolic atrial arrhythmia (65,27%), combined arrhythmias (65,93%), supraventricular paroxysmal tachycardia (71,32%) and sinus tachycardia (74,24%). SOD deficiency demonstrates the involvement of oxidative stress in cardiac arrhythmic pathogenesis, excess oxygen radicals interfering with multiple mechanisms related to the onset of arrhythmogenesis. The SOD decrease was more important in females (60,57%) than in males (67,06%) and in those with nutrition poor in antioxidants. Conclusions: SOD estimation represents a biomarker whose decrease and deficiency implies occurrence of oxidative stress and implicitly highlights its role in cardiac arrhythmic pathology in young people, with the possibility of monitoring and correction by pharmacological or non-pharmacological therapeutic means.

KEYWORDS: superoxide dismutase enzyme, oxidative stress, arrhythmia, young people

Introduction

The multitude of etiopathogenic and risk factors involved in the onset and persistence of cardiac arrhythmias, require an individualized configuration of both clinical, functional, imagistic and biochemical parametres.

If under the condition of preexisting myocardial lesions, especially ischemic type, the development of arrhythmias is predictable and morphologically motivated, the description of cardiac disturbances in healthy persons, without cardiac abnormalities, often considered as functional, involves multiple functional and biochemical elements, still not fully elucidated.

The electrophysiological mechanisms involved in arrhythmic triggering are significantly influenced by biochemical alterations, with cellular or tissue impact [1,2].

Oxidative stress, through the development and insufficient control of oxygen free radicals, in addition to inducing tissue and vascular damage, it may play a role in the installation and perpetuation of cardiac arrhythmias, by interfering with electrogenesis factors [3,4].

Superoxide-dismutase (SOD), as an important enzyme with anti-oxidant properties, may be considered as an oxidative stress biomarker and, implicitly, the estimation of its variations may explain some arrhythmic cardiac dysfunctions. The primary role of superoxide-dismutase is to dismute the superoxide radical anion, highly reactive, with harmful potential into oxygen and water [5,6,7,8].

Aim and Objectives

Determination of SOD as biomarker of oxidative stress, in healthy young subjects, diagnosed with arrhythmias, without any preexisting lesional cardiac pathology.

Objectives of the research were: to establish the diagnosis of cardiac arrhythmia (persistent or

repetitive) in normal heart, confirmed by electrocardiogram (ECG); to select patients, on age-criteria; to aquire a control group; to perform biochemical determination of serum SOD and identification of other associated factors.

Material and Methods

The study was conducted on 2 groups, consistingof young subjects, with and without cardiac arrhythmias, aged 20-45 years old, to whom the SOD value was determined.

Group I consisted of 40 patients, diagnosed with cardiac arrhythmias, 21 females and 19 males, excluding other associated diseases.

Group II, as control group, consisted of 40 healthy subjects, 30 women and 10 men, without any arrhythmic cardiac disturbances.

In the diagnosis of cardiac arrhythmias, we used: anamnesis, physical examination and paraclinical investigations (of interest being ECG and cardiac echography).

A questionnaire related to lifestyle and type of alimentation was also used, targeting a rich or poor-nutrient diet of vegetal origin (containing natural antioxidants) [6,9,10].

In all subjects, a standard blood sample was collected, in order to perform biochemical determination of SOD values and variations. In addition, other associated determinations were achieved: haemogram, urea, creatine, glycemia, ionogram, calcemia, magnesia.

Determination of SOD was performed in the biochemistry laboratory of University and Pharmacy of Craiova, using the spectrophotometric method.

Thus, dosing superoxide dismutase activity was achieved using a kit of reagents produced by Randox Laboratories, for the *in vitro* determination of SOD blood activity. The measurements were performed with a Beckman UV-VIS spectrophotometer, DU-65 model.

The obtained clinical and biochemical data were included into databases and statistically processed. We used Student's t test to compare mean values for the two groups of patients, and ANOVA, followed by Fisher's LSD post-hoc test to compare multiple groups.

Results

In group I, the average age was 34 years and in group II, 28 years.

Determination of SOD showed the following mean values: for group I: 104,63U/mL (with standard deviation-STD DEV of 18,38) and for group II: 168,97U/mL (with STD DEV of 27,57).

In percentage, mean values of SOD in young patients with arrhythmias represented 61,92% of mean SOD values in the healthy, control group, with no cardiac arrhythmias, Table 1, Fig. 1.

The recorded SOD deficit had a value of 38,08%, representing more than one third of the value expressed in the control group, Table 1, Fig. 1.

Table 1. SOD mean and percentage values variations in young patients with and without arrhythmias

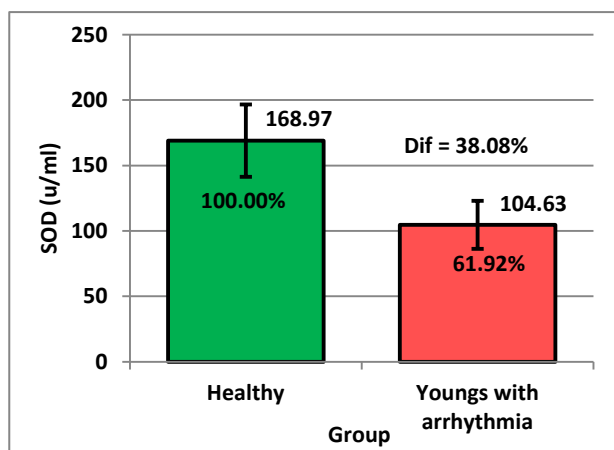| SOD-Mean Value (U/mL)		Percentage Decrease	Percentage Deficit	p value	Significance
Group I (Youngs with arrhythmias)	104,63±18.38	61,92%	38,08%	p Student <0.001	**HS**
Group II (Youngs without arrhythmias)	168,97±27.57				

Fig.1. Representation of SOD mean and percent values variations in arrhythmic patients compared to healthy subjects. Error bars represent standard deviation of the means

In terms of gender distribution, mean SOD values in females with arrhythmias were 105,11U/mL (STD DEV 22,18) and in males 104,10U/mL (STD DEV 13,6)-group I.

In the control group, the mean SOD value was 173.54U/mL for women (STD DEV 23,53) and 155.23U/mL for men (STD DEV 35,09)-group II.

In the two groups, differences between SOD reduction may be expressed, in patients with arrhythmias compared to controls, by gender; in women the SOD value represented 60,57% compared to the same-sex controls, inducing a 39,43% SOD deficiency; in men, the average SOD value compared to controls was 67,06%, with a SOD reduction by 32,94%, Table 2, Fig. 2.

Table 2. SOD-mean and percentage values variations by gender in studied subjects

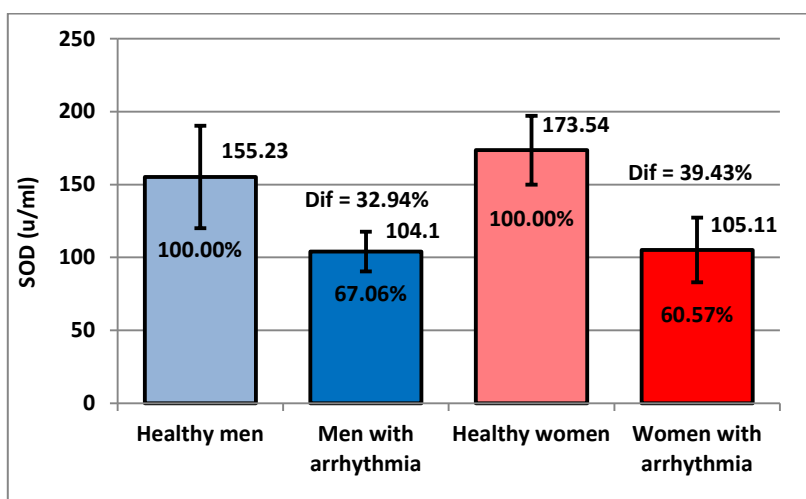| Group	SOD						
	Females p Student <0.001			Males p Student <0.001			
	Mean (U/mL)	Decrease %	Deficit %	Mean (U/mL)	Decrease %	Deficit %	
Group I (subjects with arrhythmias)	105,11±22.18	60,57%	↓39,43 %	104,10 ±13.60	67,06 %	32,94%	**p Student =0.865**
Group II (subjects without arrhythmias)	173,54±23.53	100%	0	155,23 ±35.09	100%	0	**p Student =0.068**

Fig.2. Representation of SOD mean and percent values reported by gender in patients with arrhythmias compared to healthy subjects with the same gender. Error bars represent standard deviation of the means

This may be related to the observation of arrhythmias in young subjects, more frequent in females than in males.

Cardiac arrhythmias, diagnosed by ECG, in group I of patients, were: extrasystolic atrial arrhythmia (15% of total patients with dysrhythmia), sinus tachycardia (7.5%), associated dysrhythmias (12.5%), extrasystolic ventricular arrhythmia (17.5%), paroxysmal atrial fibrillation (20%), paroxysmal supraventricular tachycardia (10%), sinus bradycardia (12.5%), atrial flutter (5%). Laboratory findings were within normal limits, not being involved or related to the arrhythmogenic process, Table 3, Fig. 3, Fig. 4, Fig. 5, Fig. 6.

Table 3. Mean and percent values of SOD in relation with arrhythmia types

| Type of arrhythmia	No. of cases (%)	SOD Mean	SOD SD	Decrease%	Deficit%
Healthy	40	168,97	27.58	100%	0
Sinus tachycardia	3 (7.5%)	125,46	9.63	74,24%	↓ 25,76 %
Paroxysmal supraventricular tachycardia	4 (10%)	120,51	21.88	71,32%	↓ 28,68%
Associated/ Multiple dysrhythmias	5 (12.5%)	111,41	18.90	65,93%	↓ 34,07%
Extrasystolic atrial arrhythmia	6 (15%)	110,30	14.31	65,27%	↓ 34,73%
Extrasystolic ventricular arrhythmia	7 (17.5%)	108,49	11.58	64,20%	↓ 35,8%
Atrial flutter	2 (5%)	94,15	5.59	55,71%	↓ 44,29%
Sinus bradycardia	5 (12.5%)	92,71	12.44	54,86%	↓ 45,14%
Paroxysmal atrial fibrillation	8 (20%)	87,10	14.29	51,54%	↓ 48,46%
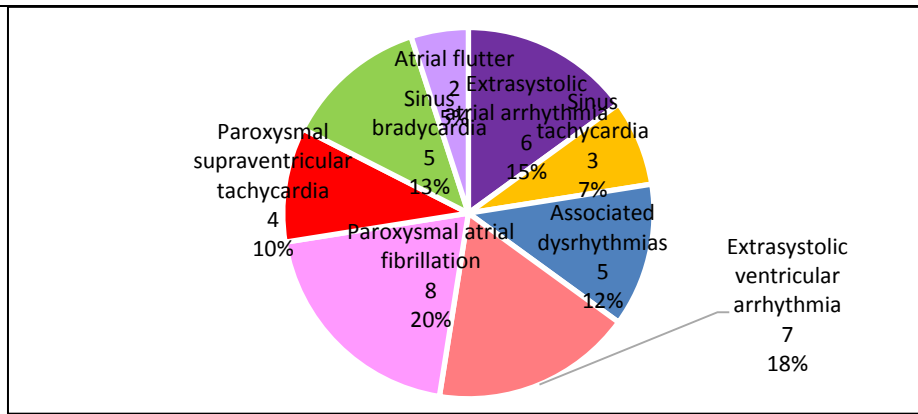
Fig.3. Percentage repartition by rhythm disorders in studied patients (group I)

Fig.4. Extrasystolic ventricular arrhythmia in a 28 years old patient with decreased SOD value
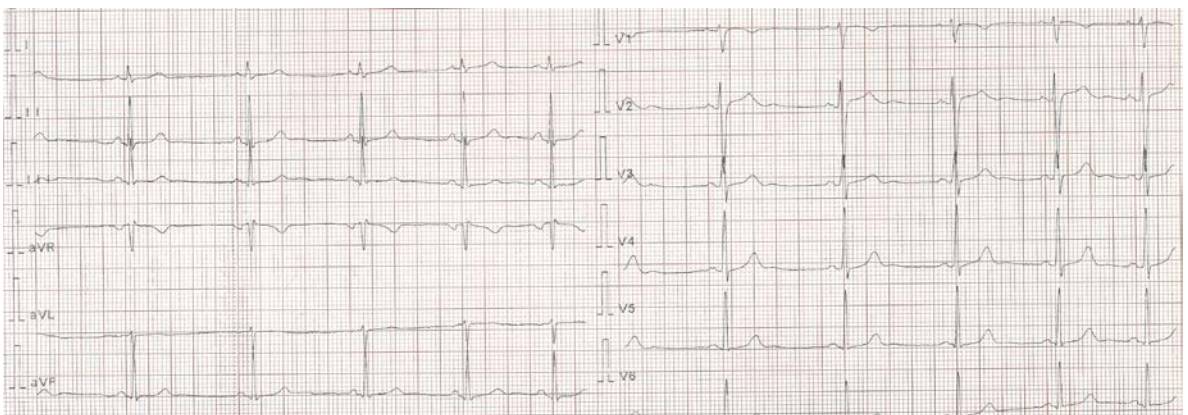

Fig.5. Sinus bradycardia in a 30 years old patient with decreased SOD value
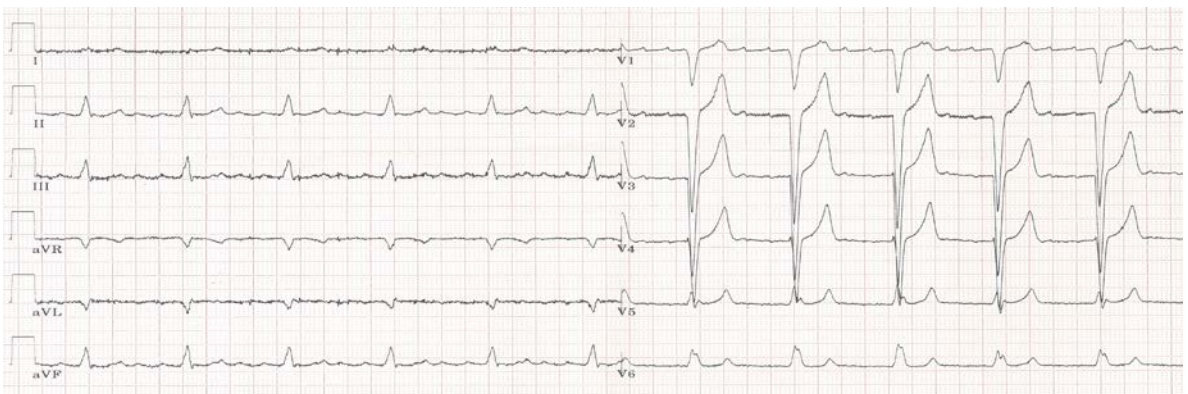

Fig.6. Atrial flutter in a 38 years old patient with decreased SOD value

In group II, ECG and echocardiography were normal, as well as the usual laboratory findings, which were within limits. The mean SOD value in these healthy subjects, without cardiac arrhythmias, was 168.97U/mL, value that was significantly higher than the mean values for all groups with arrhythmias (p ANOVA<0.001).

Depending on the type of arrhythmia, the following SOD mean values were recorded: sinus tachycardia: 125,46U/mL, paroxysmal supraventricular tachycardia: 120,51U/mL, multiple dysrhythmias 111,41U/mL, extrasystolic atrial arrhythmia 110,30U/mL, extrasystolic ventricular arrhythmia 108,49U/mL, atrial flutter 94,15U/mL, sinus bradycardia 92,71U/mL, paroxysmal atrial fibrillation 87,10U/mL, showing modifications regarding percentage expression compared to the control group and implicitly, different degrees of values decrease. Using Fisher's LCD post-hoc test, following the ANOVA test, we discovered significant differences between mean SOD values in cases with paroxysmal atrial fibrillation, cases with sinus tachycardia, and cases with paroxysmal supraventricular tachycardia.

Thus, mean values of SOD, compared to the control group, represented the following: sinus tachycardia: 74,24%; paroxysmal supraventricular tachycardia: 71,32%; multiple dysrhythmias: 65,93%; extrasystolic atrial arrhythmia: 65,27%; extrasystolic ventricular arrhythmia: 64,20%; atrial flutter: 55,71%; sinus bradycardia: 54,86%; paroxysmal atrial fibrillation: 51,54%, with a deficit of: 25,76% for sinus tachycardia; 28,68% for paroxysmal supraventricular tachycardia; 34,07% for multiple dysrhythmias; 34,73% for extrasystolic atrial arrhythmia; 35,8% for extrasystolic ventricular arrhythmia; 44,29% for atrial flutter; 45,14% for sinus bradycardia and of 48,46% for paroxysmal atrial fibrillation, Table 3, Fig. 7.
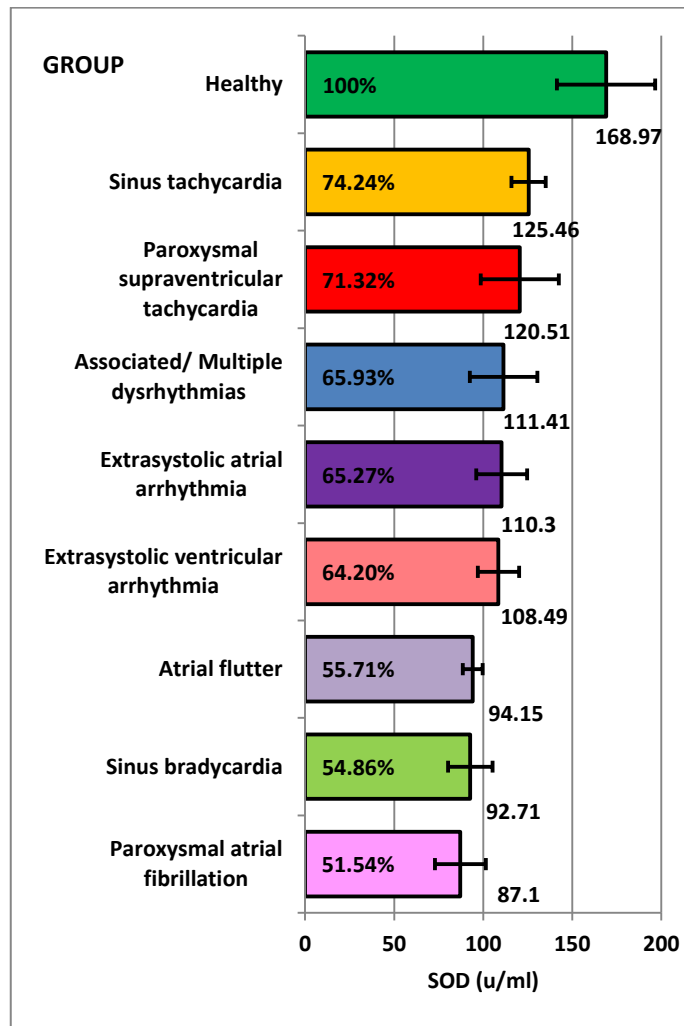

Fig.7. Representation of SOD mean and percent values in different arrhythmia types in patients compared to healthy subjects. Error bars represent standard deviation of the means

In relation to the diet-type, the following results were observed: mean value of SOD in the arrhythmic subjects group was of 117,85U/mL (STD DEV 15,35), for the ones with a predominantly vegetal diet (high in antioxidants) in comparison with the control subjects, also with this diet, with mean SOD values of 180,12U/mL (STD DEV 21,9), representing 65,43%, values which were highly significant correlated (p Student <0.001), Table 4, Fig.8.

Table 4. SOD mean value related to alimentation influence in the studied groups

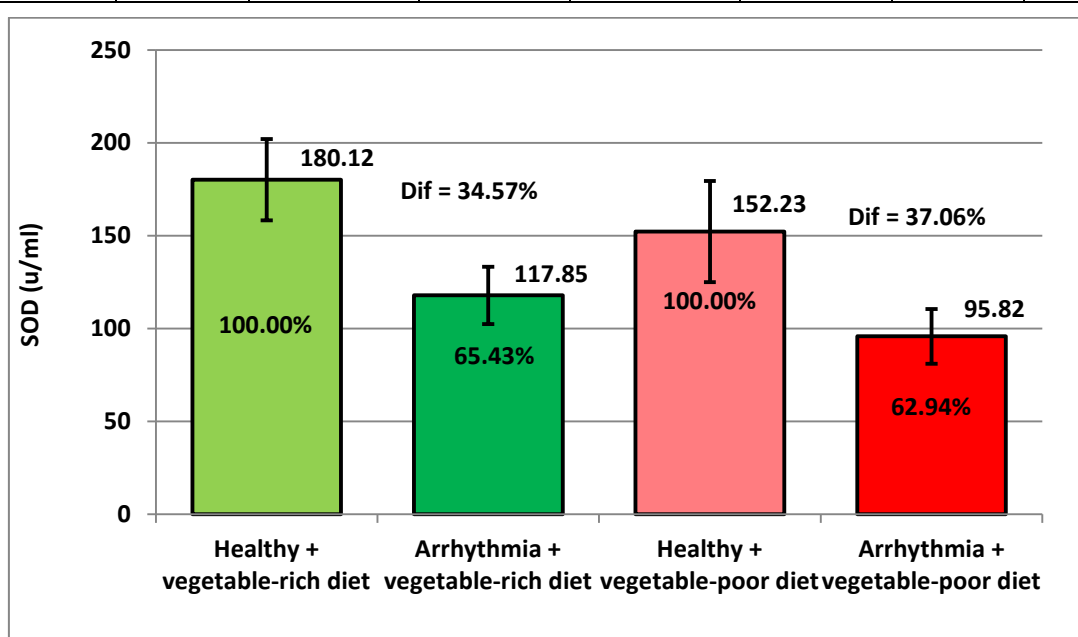| Group	SOD						
	Diet- Rich in vegetables p Student <0.001			Diet – Poor in vegetables p Student <0.001			
	Mean (U/mL)	Decrease %	Deficit %	Mean (U/mL)	Decrease %	Deficit %	
I (subjects with arrhythmias)	117,85 ±15.35	65,43%	↓34,57%	95,82±14.72	62,94%	↓37,06%	**p Student <0.001**
II (subjects without arrhythmias)	180,12 ±21.90	100%	0	152,23±27.27	100%	0	**p Student <0.001**

Fig.8. Representation of SOD mean and percentage values variations in arrhythmic patients with vegetable-rich diet and vegetable-poor diet, compared with healthy subjects with the same type of alimentation. Error bars represent standard deviation of the means

In those with poor vegetal nutrition and arrhythmias, the mean SOD value was 95,82U/mL, (STD DEV 14,72), representing 62,94% compared to the controls, with the same diet, who had a mean SOD value of 152,23U/mL (STD DEV 27,27), values being highly significant correlated (p Student<0.001), Table 4, Fig. 8.

SOD deficiency was lower in patients with predominantly vegetal nutrition, being of 34.31%, compared to the ones with low vegetal nutrition, implicitly with reduced natural antioxidants intake, to whom it was 40.06%, Table 4, Fig. 8.

Discussions

Subjects participating in the study, both those with arrhythmias, as well as the healthy ones, were young, without any cardiac or extracardiac associated lesional pathology.

SOD determination showed variations, its decrease being observed in all patients with cardiac arrhythmic disorders, compared to healthy ones, decrease which was highly statistically significant (p Student<0.001).

The enzymatic intervention of SOD variations demonstrates its role in the augmentation of free oxygen radicals, leading tothe development of an increased oxidative

stress level, that influences arithmogenesis through multiple mechanisms.

Mechanisms through which reactive oxygen species (ROS), not-neutralized by SOD generate rhythm disorders are described, as follows [11,12].

Within cardiac cells, with excitability properties, reactive oxygen species interfere both in their metabolism, as well as inthe ionic homeostasis [12].

Studies showed that arrhythmias and oxidative stress are correlated.

Either reduction, or, by the contrary, hyperfunction of natrium (Na^+),calcium (Ca^{2+}),potassium (K^+) channels, as well as alterations in the functioning of voltage-dependent ion channels, together with other mitochondrial and DNA dysfunctions, are the main ways of initiating and conduction of aberrant impulses, participating in arrhythmogenesis [11-15].

There are several types of mechanisms assumed to be involved in generating arrhythmias [11,12]:

- At myocytes level, hydrogen peroxide (H_2O_2) interferes with the action potential, prolonging its duration and inducing early or late postdepolarization, resulting in automatism disorders [12].

- H_2O_2 has a role in decreasing Na^+ currents through negative feedback on transcription of the gene that encodes Na^+ channels-SCN5A gene [16].

- Peroxidation of the lipid membrane under hydroxyl radical action has the effect of disturbing the calcium transport within endoplasmic reticulum, decreasing its capture and increasing its level in the myocytes, causing abnormal activity.

Apart from other biochemical modifications involved in the onset of cardiac dysrhythmias, the decrease of SOD, as enzyme that plays a role in the REDOX system, expresses the implication of oxidative stress and oxygen free radicals in electrophysiological processes regarding cardiac rhythm and arrhythmogenesis. This may also explain the more frequent induction of rhythm disorders such as atrial fibrillation and extrasystolic arrhythmia [17,18,19], and, along with age, the concomitent increase of oxidative stress level [4,5,7].

The decrease of SOD may represent an oxidative risk biomarker in young patients with cardiac arrhythmias; oxidative stress can induce early endothelial lesions, as well as lipid oxidation with the occurence of oxidized-LDL, leading to a faster development of atherosclerotic processes from young age [20].

Although apparently, cardiac dysrhythmia is based on ectopic reentry or ectopic focal activity, the coexistence of an oxidative stress, basically demonstrated by enzyme biomarkers, such as SOD, may indicate the risk of developing myocardial or early vascular endothelial lesions. In addition, free radicals in excess may oxidize lipid fractions, and subsequently, their oxidized state induces antibodies, which may involve pathogenic triggering of immune phenomena [21,22].

Under the conditions of SOD depletion in arrhythmic patients with nutritional diet poor in antioxidants, lifestyle modification is required, involving an increased intake of antioxidants (by nutrition or supplements) [23,24].

There have been some minor differences in the decrease of SOD by gender, which are in concordance with the more frequent description of cardiac arrhythmias in females.

Enzymatic oxidative modifications, with the occuenceof a high oxidative stress level, are significant for the arrhythmic pathology in young people.

In the investigation of young patients with arrhythmias, the determination of SOD as an oxidative stress biomarker is both a pathogenic as well as a therapeutic (pharmacological and non-pharmacological) element, that plays an important role in the early prophylaxis of subsequent degenerative processes.

Conclusions

1. SOD value in serum is an important biomarker for highlighting oxidative stress level and its etiopathogenic involvement in cardiac arrhythmias.

2. The SOD value variation recorded significant decreases in patients with non-lesional cardiac arrhythmias, of approximately 61,92% (2/3) compared to healthy subjects.

3. The 38,08% SOD deficit found in young patients with cardiac arrhythmias demonstrates that this biomarker and, implicitly, oxidative stress are involved in arrhythmogenesis.

4. The most significant deficit in SOD values was recorded, in the following arrhythmia types: 48,46% for paroxysmal atrial fibrillation; 45,14% for sinus bradycardia; 44,29% for atrial flutter; 35,5% for extrasystolic ventricular arrhythmia; 34,73% for extrasystolic atrial arrhythmia; 34,07% for multiple dysrhythmias; 28,68% for paroxysmal supraventricular tachycardia and 25,76% for sinus tachycardia.

5. SOD decrease is important in the assessment of arrhythmogenic risk in young people, and subsequently, its correction brings benefits to the major consequences of arrhythmias (sudden death, heart failure).

Acknowledgements

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Corresponding Author: Marinela Beznă, MD, PhD, Department of Internal Medicine,
University of Medicine and Pharmacy of Craiova, Petru Rares Street, No.2, Craiova, Romania;
e-mail: kris_cris2011@yahoo.com