Decrease of glutathione peroxidase in arrhythmic cardiac pathology in young individuals and its therapeutic implications

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Abstract. Glutathione peroxidase (GPx), as an antioxidant enzyme, is involved in the regulation of processes that cause cellular oxidative stress, with implications in various pathologies. The aim of the present study was to evaluate GPx variations in patients with arrhythmic, non-structural cardiac disorders. The research was performed on 120 patients, with a mean age of 33 years old, divided into 3 equal groups, of which 2 groups included patients with cardiac arrhythmias, the first group, associated with dyslipidemia and the second one, without dyslipidemia, and a control group consisting of healthy individuals. The method for determining GPx was based on the GPx enzyme catalysis reaction of the reduced glutathione (GSH) oxidation reaction by cumene hydroperoxide. The results revealed that GPx variation was decreased in patients with cardiac arrhythmias, with or without dyslipidemia, up to 66 and 74% of mean control values, respectively, the differences being statistically significant, showing the existence of an oxidative stress imbalance, that may be involved in triggering arrhythmogenic electrochemical mechanisms. The GPx deficiency determined in relation to cardiac arrhythmias was in dyslipidemic and non-lipidemic patients as follows: 29-35% in sinus bradycardia, 31-35% in associated cardiac arrhythmias, 30-33% in sinus tachycardia, 27-33% in atrial fibrillation, 32-33% in atrial flutter, 27-32% in atrial extrasystolic arrhythmia, 28-30% in ventricular extrasystolic arrhythmia and 18-26% in paroxysmal supraventricular tachycardia. Collectively, the results revealed that GPx, an antioxidant enzyme, is a specific biomarker, whose decrease indicated the existence of oxidative stress in young individuals with cardiac arrhythmias and its involvement in arrhythmogenic electrochemical processes. In addition, GPx deficiencies were between 18-35% in all types of cardiac arrhythmias, the highest being recorded in sinus bradycardia and the lowest in paroxysmal supraventircular tachycardia. Furthermore, the oxidative stress favored by the decrease of GPx induced lipid oxidation, regardless of the presence or absence of dyslipidemia, which triggered the formation of anti-lipid antibodies and a subclinical endothelial aggression, with early atherosclerotic potential. GPx evaluation may argue for the existence of oxidative stress in non-structural cardiac arrhythmias, and by its proper correction (antioxidants), prophylaxis of atherogenic dysfunction.

Introduction

Oxidative stress is involved in the pathogenesis of numerous diseases. For this reason, various methods have been developed to assess it, methods that are useful in disease research, highlighting either the degree of damage (oxidation in DNA, proteins, lipids) or the ability of the body to self-regulate and also self-defense (through feedback mechanisms, antibodies, etc.) (1,2).

Studies (1-3) have revealed that oxidative stress plays an important role in multiple heart conditions, including arrhythmias. Rhythm disorders in individuals with no pathological history are associated with the presence of systemic and cardiac oxidative stress, caused by the existence of reactive oxygen species (ROS) (1). Systematically, literature (4-6) has revealed high values of oxidative stress markers in individuals with atrial fibrillation and extrasystolic arrhythmias.

The frequency of cardiac dysrhythmias in the general population is high and occasionally, their etiology remains difficult to fully understand. Arrhythmias, in non-structural heart disease, regarded as heterogeneous pathology, worldwidely, affect 0.8-1,4% of the general population, being three times more common in females than in males. The prevalence among the general population is 1.17/1,000, with an incidence of 25/100,000 (2,3). Atrial fibrillation affects approximately 1-2% of the general population (3). Paroxysmal supraventricular tachycardia, common in young individuals, has a prevalence of 5-10% (35-55 years) (3,4). Thus, having a significant prevalence among the young population, it can be concluded that the presence of oxidative stress leads to serious health alterations (4).

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Peroxidation of the lipid membrane under the action of hydroxyl radical has the effect of disrupting the calcium transport within the endoplasmic reticulum, decreasing its uptake and increasing its level in myocytes during dystole (4,5), leading to dysrhythmias.

The harmful effect is counteracted by the existence of antioxidant systems, represented by molecules capable of neutralizing ROS, thus preventing the induction of oxidation damage (2,4). A high value of oxidative stress (determined by measuring its specific parameters), as well as a decreased total antioxidant capacity are negative prognostic factors in initiating pathological processes in the cardiovascular system (2).

To determine the activity and at the same time the cellular level of oxidative stress, it is necessary to assess specific biomarkers, namely: Superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant capacity, malondialdehyde, low-density lipoprotein (LDL)-oxidized, and anti-oxidized LDL antibodies. Their level is a predictive and prognostic factor in terms of the reactivity of the body to the agression of oxidative stress (1,2,5).

GPx represents an enzyme that possesses antioxidant activity, having a role, at the cellular level, in reducing oxidative stress. It catalyzes the reduction of organic hydroperoxides, using glutathione (GSH) (1,2), participating in the transformation ROS into compounds such as oxygen and water. The excess of ROS leads to an increased oxidative stress level, involved in various cardiovascular pathologies (2,3).

Cardiac arrhythmias are frequently described, even in young individuals; the involved factors of this etiopathogeny are usually difficult to assess and treat (4-12).

The novelty of the present research consists in the evaluation, through a measurable biomarker, GPx, of the presence of oxidative stress in young subjects, as well as its involvement in triggering non-structural arrhythmias, without any other proven etiology.

The observed GPx deficiency indicates, through the expressed oxidative stress in young individuals, the possibility of favoring cardiac arrhythmogenic mechanisms. The demonstration of oxidation, through the GPx biomarker, suggests the possibility of developing an endothelial dysfunction, by early lipid oxidation, regardless of the existence or not of a dyslipidemic status. GPx, allows the qualitative assessment of oxidative stress as well as the quantitative determination and grading of the oxidative status. The demonstration of this enzyme decrease in relation to non-structural cardiac arrhythmias and without other explicit pathogenic mechanisms, at ayoung age, allows an appropriate correction (of oxidative stress). Monitoring GPx decrease, as a parameter, also allows a therapeutic control, through antioxidant treatment. The assessment of GPx and implicitly oxidative stress can induce an increased need for antioxidants through lifestyle, dietary and therapeutic management. The improvement of GPx deficiency is thus important and novel in the monitoring and treatment of cardiac arrhythmias in young subjects, as well as for decreasing the degree of biological wear and tear (through oxidative stress). Thus, the demonstration of GPx variation, quantifiable in young individuals requires its correction, for the prophylaxis of early cardiovascular pathology onset (8,9).

The aim of the present study was the assessment of oxidative stress involvement in heart diseases, in relation to antioxidant GPx enzyme and the lipid-metabolic status.

The present research assessed the variations of the GPx enzyme, considered a biomarker of oxidative stress, in young individuals with various cardiac arrhythmias. This allowed the monitoring of the level of oxidative stress in non-structural cardiac arrhythmic pathology and also provided therapeutic guidance in relation to the use of antioxidants, in order to compensate for the recorded deficit.

GPx has been demonstrated to catalyze the reduction of organic hydroperoxides, using GSH (1,2), participating in the transformation of ROS into compounds such as oxygen and water. The excess of ROS was revealed to lead to an increased oxidative stress level, involved in various cardiovascular pathologies (2-12).

Patients and methods

Selection and ethical approval. The present research represents a prospective study, conducted on 120 human subjects, aged between 18-45 years, during a follow-up period of 4 years, divided into 3 equal groups, of 40 subjects each. Participating subjects were selected from January 2016 to January 2017 from the Cardiology and Emergency Units of the Department of Internal Medicine of Emergency Hospital and County Hospital Craiova (Craiova, Romania) and also from a private practice (Ultracord Clinic, Craiova). Healthy volunteers consisted of medical students, doctors and nurses. All the participating subjects provided their written consent (for medical investigations, blood samples, monitoring). The research was conducted in compliance with the Academic Code of Ethics, with the obtained approval (approval no. 56/19.02.2015) of the Scientific Ethics and Deontology Comittee of the University of Medicine and Pharmacy of Craiova and according to the Principles of the Declaration of Helsinki. The data obtained from these different clinics were processed within the University of Medicine and Pharmacy of Craiova, within the Departments of Biochemistry, Statistics and Informatics.

Patients. The inclusion criteria was as follows: Age 18-45 years, regardless of sex and economic and social background, Caucasian race, the presence of heart rhythm disturbances in non-structural heart disease, confirmed clinically and investigatedby electrocardiogram, Holter-ECG device, echocardiography and laboratory findings (presence/absence of dyslipidemia).

Patients with cardiac arrhythmias were divided into two groups, with and without dyslipidemia, for the homogeneity regarding this variable parameter and for its importance as a risk factor in cardiac pathology. In addition, this parameter was recorded for the study of lipid profile, considering the demonstrated role of oxidative stress, both in the induction of cardiac arrhythmias and in lipid oxidation with subsequent development of an immune response, with a pathogenic evolution towards accelerated atherosclerotic endothelial dysfunction (4).

The sex distribution of the patients was as follows: Group I, 21 women (52.50%) and 19 men (47.50%); group II, 21

Table I. Sex distribution of patients with cardiac arrythmias.

Sex distribution	Group I	Group II	Group III	No. of cases
Women	21 (52.5%)	21 (52.5%)	23 (63%)	65
Men	19 (47.50%)	19 (47.50%)	17 (37%)	55
Total	40 (100%)	40 (100%)	40 (100%)	120

Group I, patients with cardiac arrythmias with dyslipidemia; group II, patients with cardiac arrythmias without dyslipidemia; group III, healthy individuals.

Table II. Mean heights and weights of patients with cardiac arrythmias.

Groups	Mean height (cm)	Standard deviation	Mean weight (kg)	Standard deviation
Group I	169.65	10.29	79.5	17.9
Group II	170.4	8.14	71.33	12.91
Group III	168.63	8.35	70.54	14.25

Group I, patients with cardiac arrythmias with dyslipidemia; group II, patients with cardiac arrythmias without dyslipidemia; group III, healthy individuals.

(52.50%) women and 19 men (47.50%), and group III, 23 (23%) women and 17 men (17%) as presented in Table I.

As antropometric data, the mean height for group I was 169.65±10.29 cm; for group II,170.40±8.14 cm; and for group III,168.63±8.35 cm. The selected subjects had a mean weight, as follows: Group I, 79.50±17.9 kg; group II, 71.33±12.91 kg; and group III, 70.54±14.25 kg, as shown in Table II.

The exclusion criteria included the existence of systemic or visceral pathology (acute/chronic), that may be involved in cardiovascular manifestations and could alter the oxidation processess; and previous medication intake. In addition, the following exclusion criteria were applied: Young individuals with previously diagnosed pathologies, generating arrhythmias or oxidative stress including: i) pathologies that cause secondary arrhythmias such as hyperthyroidism, myxedema, anemia, hypotension, hypoglycemia, febrile syndrome, myocarditis, orpheochromocytoma; ii) pathologies that increase oxidative stress such as diabetes or other metabolic diseases (dyslipidemia, in case of group I and III), atherosclerosis (diagnosed), hypertension, autoimmune diseases, allergies, asthma, hematopoietic dysfunction, infectious diseases, oncological pathology, ulcer gastro-duodenal, acute pancreatitis and Parkinson's disease; iii) cardiovascular pathologies such as heart failure, congenital diseases and cardiovascular malformations, as well as pathologies with genetic determinism (Brugada syndrome, long QT, arrhythmogenic dysplasia of the right ventricle), chronic ischemic heart disease, hypertension, myocarditis, pericarditis, vascular disease, electric pacemaker devices; iv) non-cardiac pathologies, with severe progression, which can generate arrhythmias such as hemorrhages, various neoplasms, hematological diseases, post-surgical conditions, infectious diseases, neuropsychiatric disorders, gout, hyperuricemia, renal insufficiency, hepatic, pulmonary failure and multiorgan failure. Other miscellaneous conditions were also excluded, including excessive consumption of grapefruit, as well as possible interactions withdrug intake (antiarrhythmics, sympathomimetics, parasympatholytics, enzyme inducers, enzyme inhibitors, and antioxidants). The exclusion criteria in the control group were the same as those in the patients of the control groups (group I and II), to which the absence of cardiac arrhythmias and dyslipidemia was added.

For the control group which consisted of healthy individuals, cardiovascular manifestations and dyslipidemic status were excluded by clinical, paraclinical and biologic investigations.

Group I and II included patients who were diagnosed and monitored for non-structural cardiac arrhythmias within the profiled clinics, in the emergency units and also in the specialized private clinics. The difference between group I and II was established by the presence or absence of dyslipidemic status, as a risk factor, associated with cardiac pathology. Thus, group I included patients with cardiac arrhythmias and dyslipidemia, group II included patients with cardiac arrhythmia disorders but without dyslipidemia, and group III consisted of healthy subjects.

Methods. Subjects were evaluated clinically and paraclinically for evidence of cardiac arrhythmic disorders, for dyslipidemic status, and in particular, GPx values were assessed. On all subjects, the following tests were performed: ECG, Holter-ECG monitoring, echocardiography and general ultrasound, cardiology consultation, usual biochemistry analysis, lipidogram and oxidative stress testing, by determining GPx values. In all subjects, in addition to the usual tests (blood glucose, complete blood count, creatinine, aspartate and alanine transaminases), lipidograms were assessed, in order to determine the lipid-metabolic status.

GPx enzyme determination method. The activity of the antioxidant enzyme GPx was determined by standard protocols, in blood samples (2 ml each), collected from all subjects,

Table III. Mean and percentage values of GPx inpatients with cardiac arrythmias, compared with healthy individuals.

Groups	No. of subjects	Mean (mmol/l)	Standard deviation	Percent (%)	Difference (%)
Group I	40	404.59	39.27	68.10	31.90
Group II	40	437.11	48.58	73.57	26.43
Group III	40	594.13	88.07	100.00	0.00

Group I, patients with cardiac arrythmias with dyslipidemia; group II, patients with cardiac arrythmias without dyslipidemia; group III, healthy individuals.

after the onset of arrhythmia (for groups I and II), by venipuncture (in K^2EDTA vacutainers), using a standardized Ransel Laboratory kit (RandoxLaboratories, Ltd.). Hemolysates were used as blood biological samples. The values were assessed using a Beckman DU-65, UV-VIS type, spectrophotometer, at the Department of Biochemistry of the University of Medicine and Pharmacy of Craiova.

According to the substrate used for the oxidation of GSH, both forms of GPx (seleno-dependent and non seleno-dependent) can react. In blood, the erythocytes contain only the seleno-dependent isoenzymes, while plasma contains 80% of the seleno-dependent form and 20% of the non seleno-dependent form. GPx1 is the predominant isoform and is expressed in the heart. In the present research, GPx1, as the most widely distributed and abundant in human cells (3,9,11,12), was studied.

The principle of the method describes the catalysis by the GPx enzyme of the oxidation reaction of reduced GSH by cumin hydroperoxide. The resulting oxidized GSH is rapidly converted to its reduced form, triggering the oxidation of NADPH to the NADP$^+$ form, in the presence of glutathione reductase and NADPH (3,9). The absorbance decrease is measured at 340 nm. The reference values are 27.5-73.6 U/gHb or 4,171-10,881 U/l. Mean GPx values were calculated for the studied groups, compared and observed in association with cardiac arrhythmias, and the lipidogram results (presence or absence of dyslipidemia).

Statistical analysis. The obtained data were recorded and processed statistically at the Department of Statistics and Informatics of the University of Medicine and Pharmacy of Craiova. The data was then interpreted and discussed, included into specific databases, where correlations and statistics were performed, in order to obtain conclusions concerning the GPx enzyme as a biomarker of oxidative stress and objective therapeutic indications for reducing the level of oxidative stress and respectively cellular oxidation. For these purposes, Microsoft Excel (Microsoft Corp.), XLSTAT 2014 for MS Excel (Addinsoft SARL) and IBM SPSS Statistics 20.0 (IBM Corporation) programs were used, thus creating databases used in the present research (3,9,11).

The most significant statistical parameters used were: Mean and standard deviation, analysis of variance (ANOVA for analyzing the dispersion of a numeric variable, under the influence of a grouping variable) and Fisher LSD post hoc tests (to identify the pairs of groups between which differences were found). Graphic representation was achieved using the
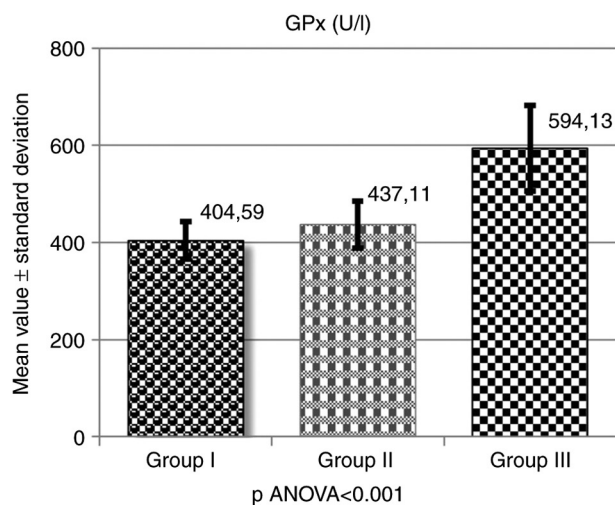

Figure 1. GPx mean values compared with controls, by groups. For group I the mean values of GPx were 404.59±39.27 U/l-hemolyzed; for group II, 437.11±48.58 U/l-hemolyzed; and for group III, 594.13±88.07 U/l-hemolyzed. ANOVA test revealed significant differences between group I and group III and between group II and group III. Group I, patients with cardiac arrythmias with dyslipidemia; group II, patients with cardiac arrythmias without dyslipidemia; group III, healthy individuals. GPx, glutathione peroxidase.

MS Excel program, with the following functions: Pivot Tables, Functions, Statistical, Chart, and Data. All the information were graphically represented using the following statistical indicators: Mean, standard deviation, using ANOVA and Fisher LSD post hoc tests. When the ANOVA test presented a statistically significant result, it was further analyzed using Fisher LSD post hoc test, in order to assess the groups between which the statistically significant differences were obtained (3,9,11).

Results

GPx assessment was conducted by calculating the mean values obtained within the three groups. Thus, for group I (patients with cardiac arrhythmias and dyslipidemia), the mean value of GPx was 404.59±39.27 U/l-hemolyzed; for group II (patients with cardiac arrhythmias without dyslipidemia), the mean value of GPx was 437.11±48.58 U/l-hemolyzed; and for group III, healthy controls (no arrhythmias, no dyslipidemia), the mean value was 594.13±88.07 U/l-hemolyzed (Table III and Fig. 1).

The mean values of GPx were decreased in the two groups (I and II), compared with the control (group III). For group I, a GPx deficit of 31.90% was registered, the mean values

Table IV. GPx mean and percentage values, according to types of arrhythmias in group I.

Arrhythmia types in group I	Mean value (mmol/l)	Percent (%)	Standard deviation	No. of cases
GPx (U/l) group III	594.13	100	88.07	40
Paroxysmal supraventricular tachycardia	439.04	73.90	76.47	5
Extrasystolic ventricular arrhythmia	416.29	70.07	52.00	5
Atrial extrasystolic arrhythmia	403.63	67.93	14.92	4
Atrial flutter	402.14	67.69	0.00	2
Atrial fibrillation	399.85	67.30	23.77	10
Sinus tachycardia	396.95	66.81	29.36	8
Associated (multiple) cardiac dysrhythmias	387.17	65.17	42.67	3
Sinus bradycardia	384.18	64.66	22.61	3

Group I, patients with cardiac arrythmias with dyslipidemia; group III, healthy individuals.

being decreased compared with the control group, at 68.10%. In group II, the GPx deficit compared with the healthy individuals was 26.43%, with the mean values being decreased up to 73.57%.

The deficiency of GPx and the decrease of the GPx mean values in relation to the control group, by types of arrhythmia, noted in each group, included: Group I, paroxysmal supraventricular tachycardia: 439.04±76.47 U/l-hemolyzed, with a decrease up to 73.90% compared with the control group, and an enzyme deficiency of 26.1%; extrasystolic ventricular arrhythmia: 416.29±52 U/l-hemolyzed, representing 70.07%, compared with the control and with a deficit of 29.93%; atrial extrasystolic arrhythmia, the calculated mean value of GPx was 403.63±14.92 U/l-hemolyzed, the reduction being 67.93%, compared with the control, registering a deficit of 32.07%; atrial flutter: GPx had a mean value of 402.14 U/l-hemolyzed, representing 67.69% and a deficit compared with the control of 32.31%; atrial fibrillation: 399.85±23.77 U/l-hemolyzed, being decreased up to 67.30% compared with the control, with a deficit of 32.70%. In sinus tachycardia, the recorded mean value of GPx was 396.95±29.36 U/l-hemolyzed, representing 66.81% of the control value and an enzyme deficiency of 33.82%; in combined arrhythmias: GPx exhibited a mean value of 387.17±42.67 U/l-hemolyzed, representing 65.17%, with a GPx enzyme deficiency of 32.31%; in sinus bradycardia, the recorded mean value of GPx was 384.18±22.61 U/l-hemolyzed, the decrease of GPx being up to 64.66% and a deficit of 35.34%, compared with the control (Table IV and Fig. 2).

In group II, for the patients with arrhythmias, without dyslipidemia, the mean values of GPx decrease and enzyme deficiency, by types of arrhythmias included sinus tachycardia: 416.88 U/l-hemolyzed, representing 70.17% of the control value and an enzyme deficiency of 29.83%; combined arrhythmias: 411.13±25.85 U/l-hemolyzed, representing 69.20%, with a GPx enzyme deficiency of 30.80%; paroxysmal supraventricular tachycardia GPx exhibited a mean value of 489.37±35.11 U/l-hemolyzed, with a decrease of up to 82.37% compared withthe control group, and an enzyme deficiency of 17.63%; extrasystolic ventricular arrhythmia: 430.70±59.33 U/l-hemolyzed, representing 72.49%, compared with the control and with a deficit of 27.51%; in atrial extrasystolic arrhythmia, the calculated mean value was
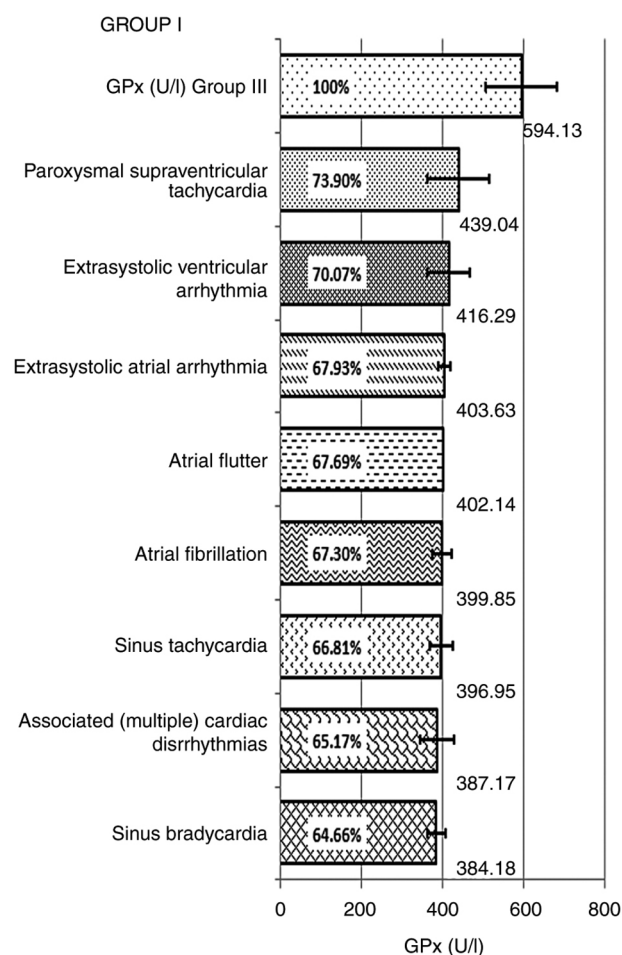

Figure 2. GPx mean and percentage values of decrease according to types of arrhythmias in group I, patients with arrhythmias and dyslipidemia compared with group III, healthy individuals. GPx had the following mean values in: Paroxysmal supraventricular tachycardia, 439.04±76,47 U/l-hemolyzed; extrasystolic ventricular arrhythmia, 416.29±52.00; atrial extrasystolic arrhythmia, 403.63±14.92; atrial flutter, 402.14±0.00; atrial fibrillation, 399.85±23.77; sinus tachycardia, 396.95±29.36; combined arrhythmias, 387.17±42.67; and sinus bradycardia, 384.18±22.61. GPx, glutathione peroxidase.

436.60±45.28 U/l-hemolyzed, the reduction being 73.48%, compared with the control group, recording a deficit of

Table V. GPx mean and percentage values, according to types of arrhythmias in group II.

Arrhythmia types in group II	Mean value (mmol/l)	Percent (%)	Standard deviation	No. of cases
GPx (U/l) group III	594,13	100	88,07	40
Paroxysmal supraventricular tachycardia	489,37	82,37	35,11	6
Atrial extrasystolic arrhythmia	436,60	73,48	45,28	11
Atrial fibrillation	432,23	72,75	42,15	7
Extrasystolic ventricular arrhythmia	430,70	72,49	59,33	6
Sinus bradycardia	423,65	71,31	64,00	4
Sinus tachycardia	416,88	70,17	0,00	1
Associated (multiple) cardiac disrhythmias	411,13	69,20	25,85	3
Atrial flutter	395,56	66,58	7,90	2

Group II, patients with cardiac arrythmias without dyslipidemia; group III, healthy individuals.

26.52%. GPx had mean values of 395.56±7.90 U/l-hemolyzed in atrial flutter, representing 66.58% and a deficit compared with the control of 33.42%; in atrial fibrillation the GPx mean value was 432.23±42.15 U/l-hemolyzed, being decreased up to 72.75% compared with the control group, with a deficit of 27.25%. In sinus bradycardia, the recorded mean value was 423.65±64.00 U/l-hemolyzed, the decrease of GPx being up to 71.31% and a deficit of 28.69%, compared to the control group (Table V and Fig. 3).

In regard to the statistical analysis, the ANOVA test revealed statistically significant differences between the mean values of GPx, with a $P<0.001$. The Fisher LSD posthoc test, used to identify the pairs of groups between which there were differences, showed significant differences between the group with arrhythmias and dyslipidemia (group I) and the control group, as well as for group II, with arrhythmia without dyslipidemia and the control group. The decrease in the GPx enzyme, considered an oxidative stress biomarker, indicates the need to administrate antioxidant therapy for the treatment and prophylaxis of non-structural heart rhythm disorders in young individuals without other associated pathology.

The analysis of the sex distribution in the three groups, revealed no statistically significant differences.

Regarding the antropometric data, no significant statistical differences were found between the mean heights of the subjects in the three study groups (ANOVA test, $P=0.531$).

For the mean weight, ANOVA test revealed that there were significant differences between the three groups ($P=0.005$). Furthermore, Fisher LSD post hoc test was used to identify the pairs of groups where differences were found and the results revealed that there was a significant difference between group I and group III.

Discussion

The GPx enzyme allows the assessment of oxidative status, by determining its values, in different situations, both normal and pathological. This enzyme has an antioxidant role, against the oxidative transformation (oxidation) of GSH (5).

Structural heart disease has also been defined according to presence/absence of dyslipidemia due to the fact that this metabolic condition is an important factor in assessing the risk
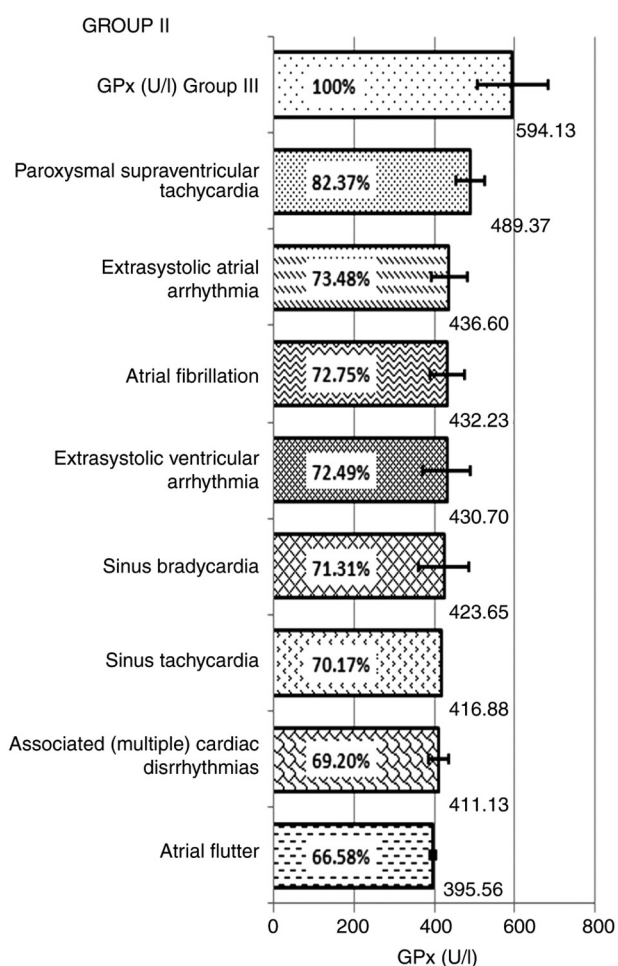

Figure 3. GPx mean and percentage values of decrease according to types of arrhythmias in group II, patients with arrhythmias without dyslipidemia compared with group III, healthy individuals. GPx had the following mean values in: Sinus tachycardia, 416.88±0.00 U/l-hemolyzed; combined arrhythmias, 411.13±25.85; paroxysmal supraventricular tachycardia, 489.37±35.11; extrasystolic ventricular arrhythmia, 430.70±59.33; atrial extrasystolic arrhythmia, 436.60±45.28; atrial flutter, 395.56±7.90; atrial fibrillation, 432.23±42.15; and sinus bradycardia, 423.65±64.00. GPx, glutathione peroxidase.

of cardiovascular disease. It represents a trigger for cardiac and vascular pathologies (5,10).

In young patients with non-structural cardiac arrhythmias, a statistically significant decrease (P<0.001) of this enzyme was found, compared with healthy subjects. The mean GPx values showed a decrease in all patients with cardiac arrhythmias, regardless of the lipid status (with or without dyslipidemia).

A decrease of up to 68.10% of the total GPx value (with mean values of 404.59±39.27 U/l-hemolysed), in patients with cardiac arrhythmias and dyslipidemia and up to 73.57% (with mean values of 437.11±48.58 U/l-hemolyzed), in those with arrhythmias, without dyslipidemia, revealed the existence of oxidative stress in these patients, by reducing the antioxidant level. According to the types of arrhythmias, GPx exhibited variations, with all arrythmias decreasing, up to 64.66% compared with the control group.

According to arrhythmic profile, the slightest decrease was exhibited in paroxysmal supraventricular tachycardia, for both groups (73.90% for patients in group I and 82.37% for group II) and the most significant decrease was revealed in sinus bradycardia (64.66%), in the dyslipidemia group and also in atrial flutter (66.58%) in non-dyslipidemics, all compared with the control group. GPx, as a biomarker, demonstrated, by its decrease, in cardiac arrhythmic pathology, the existence of oxidative stress, which can influence the electrochemical processes generating non-structural arrhythmic pathophysiological disorders in young individuals (2,3,11-25).

Antioxidant enzyme deficiencies, for dyslipidemic patients, were: 35% for sinus bradycardia and associated arrhythmias, 33% for sinus tachycardia, 33% for atrial fibrillation, 32% for atrial flutter and atrial extrasystolic arrhythmia, 30% for extrasystolic and ventricular arrhythmia, and 26% for paroxysmal supraventricular tachycardia. In addition, for non-dyslipidemic patients the antioxidant enzyme deficiencies were: 33% for atrial flutter, 31% for various combined arrhythmias, 30% for sinus tachycardia, 29% for sinus bradycardia, 28% for ventricular extrasystole, 27% for atrial fibrillation and atrial extrasystole, and 18% for paroxysmal supraventricular tachycardia.

The decrease of GPx was obtained by testing, and the decreased values of approximately 1/3 (30-35%) are significant for assessing the deficit level and the influence of increased oxidative stress, in the absence of other determined mechanisms. This may be an indicator of a high level of oxidative stress in non-structural cardiac arrhythmias. In cardiac rhythm disorders, regardless of type, electrical and biochemicalchanges may influence the sensitivity of the myocardial structure in triggering different arrhythmias. A decrease of GPx of 30-35% may be an indicator for the level of risk, favoring a cardiac arrhythmia.

A previous study by Niki (26) demonstrated that there is a strong correlation between arrhythmias and the oxidative stress status. An imbalance (excess/reduction) of K^+, Na^+-channels (encoded by the SCN5A gene), Ca^{2+}, as well as ion channel disturbances, together with various DNA and mitochondrial alterations represent the most important triggers in initiation and abnormal nervous conduction, thus leading to arrhythmogenesis. In addition, through various experimental studies, it was shown that ROS may trigger cardiac ectopic activity (25,26), because they affect (prolong) the action potential duration, causing early but also delayed post-depolarization and thus, the activity of aberrant fascicles.

The increase of ROS within a high oxidative stress level (by decreasing of the antioxidant GPx enzyme) increases the possibility of lipid oxidation, especially LDL-cholesterol and thus favors the formation of anti-LDL-oxidized cholesterol antibodies, which will lead to the initiation of an immune and vascular aggression process (13-18).

Lipid oxidation, through excess ROS (increased oxidative stress), marked by decreased GPx may occur in both patients with arrhythmias and dyslipidemia, which will result in endothelial damage with early initiation of atherosclerosis (14-17).

As sensitive biomarker for the evaluation of oxidative stress, GPx is a landmark in the indication of the therapeutic combination of antioxidants until the normalization of this enzyme and the reduction of electrochemical processes leading to cardiac arrhythmias in young patients, and also until the decreased risk of early lipid oxidation (normal or dyslipidemic profile) and for the prophylaxis of early atherosclerotic endothelial processes (27-32). Patients monitoring was performed through regular controls and treatment, specifically antioxidant medication.

In conclusion, it was revealed that i) the antioxidant enzyme, GPx, is a biomarker which indicates the existence of subclinical oxidative stress, with pathological implications; ii) in non-structural cardiac arrhythmias in young individuals, GPx exhibited lower values compared with the healthy control group; iii) the decrease of GPx observed in cardiac arrhythmic pathology was up to 2/3 (64.66%), showing the presence of oxidative stress; iv) oxidative stress maybe involved in arrhythmogenic electrochemical processes; v) regardless of the presence or absence of dyslipidemia, the oxidative stress status, highlighted by the decrease of GPx, triggers lipid oxidation, with the formation of anti-LDL-oxidized antibodies, leading to the onset of vascular endothelial aggression, with early atherogenic pathology; and vi) GPx assessment is important in objectifying oxidative stress in non-structural cardiac arrhythmias and their treatment, as well as in preventing the onset of atherosclerosis at a young age.

Availability of data and materials

The datasets used and analyzed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

MCB, CP, MB, SD, IRT, CEN and PRM contributed equally to the patient consultations and follow-up, as well as the data analysis and writing of the manuscript. The study was conceived by MCB, CP, MB and PRM. MCB and PRM confirm the authenticity of all the raw data. All authors approved the final version of this manuscript.

Ethics approval and consent to participate

The research was conducted in conformity with the Universitary Code of Ethics, and the approval (approval no. 56/19.02.2015) of the Scientific Ethics and Deontology Comittee of the University of Medicine and Pharmacy of Craiova and according to the Principles of the Declaration of Helsinki (European Union Guidelines). All the participating subjects provided their written consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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