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Death-causing cardiac injuries after chronic alcohol intake identified by forensic medicine

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Abstract

Ethyl alcohol is the most consumed drug, worldwide, with frequent consequences on the individual's health and lifestyle. Chronic alcoholism is a pathological state occurring after an excessive alcohol intake and may be observed in teenagers or very old individuals. The study performed by us investigated the changes caused by alcohol intake in the left ventricle myocardium in 77 bodies deceased at home under suspect circumstances and sent to the Institute of Forensic Medicine for establishing the cause of death. In all the individuals, there was determined high levels of blood Ethyl glucuronide, thus showing the alcohol intake up to 96 hours before death. The lesions present in the heart were represented by dilated cardiomyopathy, myocardial fibrosis, and myocardial infarction.

Keywords: chronic alcohol intake, cardiac changes, Ethyl glucuronide, blood.

Introduction

Alcohol or Ethanol (Ethyl alcohol) is the most used drug worldwide [1], while the intake rate is ever-growing, especially in teenagers and young people [2]. Some studies show that during 2016 the alcohol intake led to 2.8 million deaths all over the world, and caused numerous conditions or disabilities in individuals aged between 15 and 49 years old [3].

Although some epidemiological studies suggested that there could be some possible benefits from a low and controlled alcohol intake, in the last decades the studies indicated that alcohol has a toxic effect on the human body [4]. Moreover, alcohol is a product with immuno-suppressing, proinflammatory and pro-oncogenic effects [5, 6].

Alcohol abuse, due to the medical consequences it generates, represents an important public health issue [7–9]. The study of existing protocols in identifying the markers of chronic alcohol intake performed in laboratories in Romania and other countries showed that alcohol directly affects the most important organs and tissues, leading to systemic dysfunctions, which can have severe health consequences, from a declining quality of life to death [10–14]. Chronic alcohol users may experience significant functional and morphological changes [15–17]. In a long term, all tissues and organs are vulnerable to alcohol abuse, but almost all medical consequences are only partially reversible with abstinence [18–20].

Twenty-five percent of chronic alcohol users have a form of cardiovascular disease. The pathogenesis is mainly caused by the toxicity of alcohol on the striated muscle fiber, leading to inflammation of the heart muscle, cardio-myopathy, arrhythmias, and abnormalities of the left ventricle [21].

In addition to the direct toxic effect, there is a secondary impairment, resulting from alcohol-induced hypertension and changes in blood lipids that significantly contribute to cardiovascular morbidity. A slight increase in blood pressure can occur with moderate alcohol intake. An amount of 1 g/kg body weight/day of alcohol for five days is sufficient for a significant increase in blood pressure, especially in people who are already hypertensive. Hyper-tension occurs frequently in alcohol withdrawal, even in those without a history of hypertension, and can be a serious complication [22].

Coronary artery disease is six times more common in alcoholics and causes a 20% higher mortality rate. All heart problems and electrocardiographic abnormalities are reduced during alcohol withdrawal [23].

Aim

The present study proposes to examine the forensic cases of the Institute of Forensic Medicine (IFM), Iaşi, Romania, and to select cases with a history of chronic alcohol intake: (*i*) select the deceased cases, alleged victims of chronic Ethanol intake; (*ii*) quantify Ethyl glucuronide (EtG), a biomarker that is specific to chronic alcohol intake, in blood samples collected from the corpse, using gas-chromatographic methods; (*iii*) correlate the micro- and macroscopic organic lesions with the level of EtG detected in blood samples.

Materials and Methods

The study was performed on 77 corpses, at the IFM Iaşi morgue, between June 15, 2020 and December 20, 2020, according to the Approval No. 9421/12.06.2020 issued by the Research Ethics Committee within Grigore T. Popa University of Medicine and Pharmacy, Iaşi.

The survey data show that the patients included in the study were chronic consumers of alcohol.

The following cardiac lesions were found macroscopically during the necropsy examination: left ventricular hypertrophy, dilated cardiomyopathy (DCM), myocardial fibrosis, myocardial infarction.

For the histopathological (HP) examination, from all the individuals included in the study, there were harvested fragments of myocardium from the left ventricle, with a surface of approximately 2/2 cm, comprising all the thickness of the ventricular wall, from the epicardium to the endocardium; these were immediately fixed in 10% neutral buffered formalin for 48 hours. Then, there were harvested fragments with a thickness of 1–2 mm, subsequently subjected to the process of histological paraffin inclusion, according to the classical HP protocol. After the paraffin inclusion, there were performed 4 μm thick sections at the microtome, subsequently stained with Hematoxylin–Eosin (HE) and the Goldner–Szekely (GS) trichrome. For the immunohistochemical (IHC) study, from the same biological material (left ventricle wall), there were performed sections at the microtome that were laid on poly-L-lysine slides and then subjected to some immunostaining processes, to bring new details on the microscopic lesions of the myocardium in Ethanol consumers.

In our study, we used the following antibodies: anti-desmin (monoclonal mouse anti-human, clone D33, 1/50 dilution, Dako); anti-cluster of differentiation (CD) 68 (monoclonal mouse anti-human CD68, clone KP1, 1/100 dilution, Dako); anti-CD3 (monoclonal mouse anti-human CD3, clone F7.2.38, 1/25 dilution, Dako); anti-CD20 (monoclonal mouse anti-human CD20cy, clone L26, 1/50 dilution, Dako); anti-CD34 (monoclonal mouse anti-human CD34 Class II, clone QBEnd/10, 1/50 dilution, Dako).

The final cause of death of those mentioned was acute cardio-respiratory failure, which occurred because of cardiac lesions.

The data were centralized in a Statistical Package for the Social Sciences (SPSS) 18.0 database and processed with the statistical functions for which they are suitable, at the significant threshold of 95%.

We used the analysis of variance (ANOVA) test for evaluating the following descriptive statistical indicators: minimum, maximum, average, median, standard deviation, standard error of the mean, variance. The Skewness test ($-2<p<2$) validates the normality of the value series. In calculating the significant difference between two means, Student's *t*-test considers the measurement of variability and the weight of observations for series of values with normal distributions. χ^2 (*Chi*-squared) is a nonparametric test comparing two or more frequency distributions from the same population, applied when expected events are excluded.

The blood samples from the cases studied were harvested at a maximum time of four hours after death, for determining the level of EtG by using the gas-chromatographic method.

To highlight the systemic changes of the organ, we analyzed macro- and microscopic samples of tissues taken from the heart.

Results

The studied group consisted of 77 corpses examined in the IFM Iaşi, chronic alcohol consumers, aged between 18 and 89 years old. Similar to other studies, we also divided our group according to age: younger and older than 60 years old. We observed that a number of 46 (59.7%) individuals deceased due to alcohol abuse were aged between 60 and 89 years old, while 31 (40.3%) individuals were adults aged between 18 and 60 years old. Regarding the sex distribution, we observed that 57 (74%) of the deceased individuals were men and 20 (26%) were women. Social environment distribution showed us that 49 (63.6%) individuals lived in the rural area and only 28 (36.4%) came from the urban area (Table 1; Figure 1).

Table 1 – ***Descriptive demographic data***

Demographic characteristics	*n*=77
Average age ± SD [years] (min.–max.)	61.95±14.16 (18–89)
≥60 years old, *n* (%)	46 (59.7%)
Males, *n* (%)	57 (74.0%)
Rural, *n* (%)	49 (63.6%)

n: No. of cases; SD: Standard deviation.

The examination of the heart during necropsy in alcohol consumers showed, in all subjects, increases of the heart size, especially of the left ventricle, with hypertrophy or atrophy of the myocardium, and expansion of the left ventricle, heart failure changes, myocardial fibrosis and aortic atherosclerosis. In nine patients, there were identified lesions specific to myocardial infarction. The macroscopic examination concluded that 62 (80.5%) subjects presented DCM, 71 (92.2%) subjects presented myocardial fibrosis, while nine (8.2%) individuals presented myocardial infarction.

In all the patients included in the studied group, there was determined the blood level of EtG (a product of Ethanol metabolization) through gas-chromatographic method. The highest values of the metabolite were observed in the patients with myocardial infarction (845 ng/mL), DCM (726 ng/mL), while the lowest values were found in individuals with myocardial fibrosis (619 ng/mL). Student's *t*-test was positive (p=0.001) (Table 2; Figure 2). The presence of this metabolite confirmed the clinical data regarding the alcohol intake of the subjects included in the study.

Table 2 – ***The average level of EtG in the blood depends on cardiovascular complications***

Complication	*n* (%)	Concentration of EtG [ng/mL]	Mean/ min.–max. [ng/mL]	Student's *t*-test *p*
Dilated cardiomyopathy	62 (80.5%)	726±203	1131 / 9–7384	*0.001*
Myocardial fibrosis	71 (92.2%)	619±178	974 / 9–7384	*0.001*
Myocardial infarction	9 (8.2%)	845±638	1914 / 9–5902	*0.001*

EtG: Ethyl glucuronide; *n*: No. of cases.

The HP and IHC studies performed on the myocardium fragments harvested from the left ventricle highlighted significant microscopic changes of the myocardial muscle cells, but also of the heart.

In the patients diagnosed with DCM, the myocyte

damaging was completely heterogeneous, therefore, the cells, in classical stainings, appeared more or less colored, a state showing an important change of the sarcoplasmic content of the cells, mainly of the contractile and non-contractile proteins, as well as of glycogen. Some cells presented an amorphous sarcoplasm, where there were no highlighted myofibrils and the transversal striation, characteristic of cardiac muscular fibers, was absent. Quite frequently, in the sarcoplasm, there were highlighted vacuoles of various sizes (Figure 3a).

In some subjects, there was highlighted an intense process of myolysis, apoptosis and necrosis, with numerous muscular cells partially or completely damaged and with high quantities of cellular debris in the elements of the conjunctive matrix (Figure 3, b and c). In other subjects, there was observed the presence of some hypertrophied myocardial fibers, with clear myofibrils in the sarcoplasm (Figure 3d).

The conjunctive matrix of the myocardium in patients with DCM appeared more highlighted, with large spaces in the muscular fibers, occupied with moderate interstitial edema, fibroblast conjunctive cells and collagen fibers. Most patients presented a moderate collagenous, mainly interstitial fibrosis (Figure 3e).

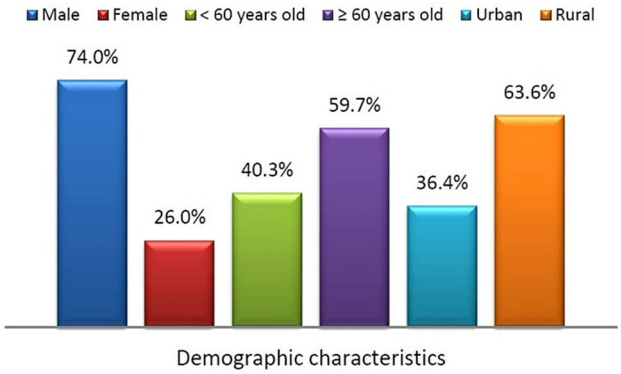

Figure 1 – ***Demographic characteristics of the studied batch.***

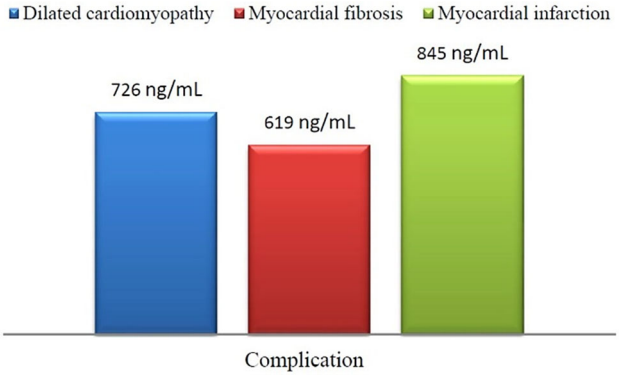

Figure 2 – ***Mean levels of Ethyl glucuronide in the blood depending on the associated cardiovascular disease.***

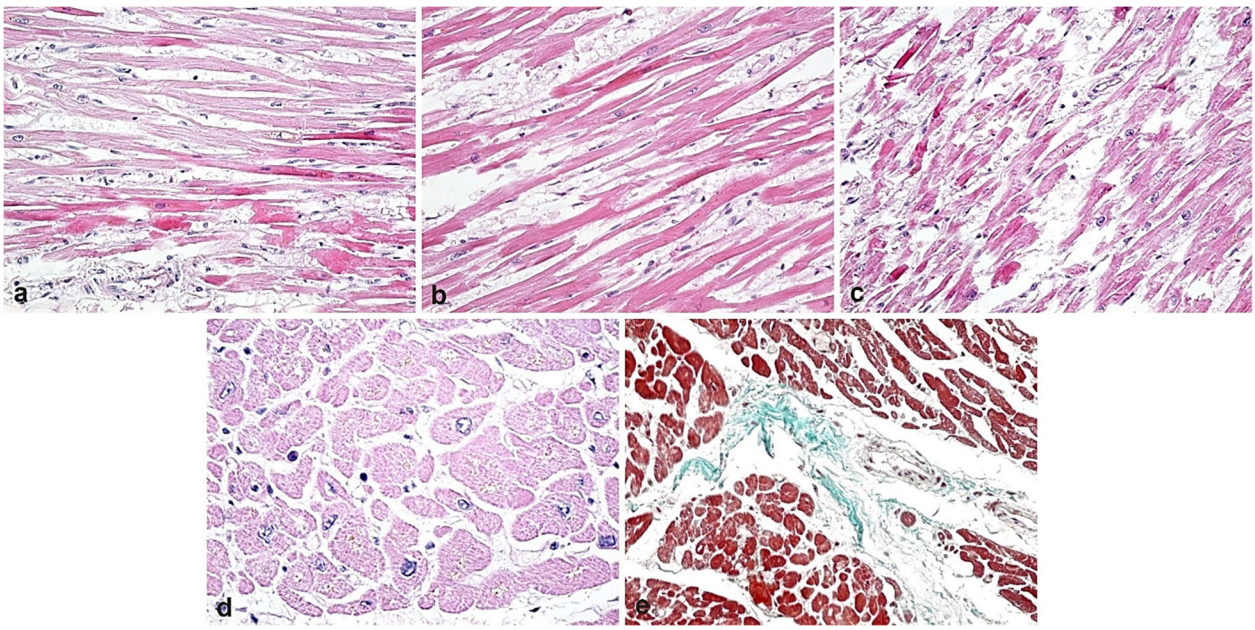

Figure 3 – ***Microscopic images from subjects diagnosed with DCM: (a) Microscopic image of myocardial fibers in a longitudinal section, with a heterogeneous coloring, with amorphous sarcoplasm cells, with an absent characteristic transversal striation; (b) Area of myocardium with numerous cells partially or completely lysed through necrosis or apoptosis; (c) Extended area of myocyte necrosis; (d) Myocardial, hypertrophied fibers in transversal sectioning, with thickened myofibrils; (e) DCM with moderate interstitial fibrosis. HE staining: (a–c) ×200; (d) ×400. GS trichrome staining: (e) ×200. DCM: Dilated cardiomyopathy; GS: Goldner–Szekely; HE: Hematoxylin–Eosin.***

The IHC examination of the myocardium from subjects diagnosed with DCM was performed to complete the HP information regarding myocardial changes. Immunomarking with the anti-desmin antibody showed that, in chronic alcohol consumers, myocytes contain low, variable desmin quantities, a protein found in the structure of intermediary filaments of *Z* stria and Eberth intercalated disk (Figure 4, a and b). In its turn, the *Z* stria regulates the sarcoma architecture, being the connection element between myofibrils, sarcomeres, and sarcolemma. The reduction of the desmin quantity in cardiac muscular fibers causes a reduction of the contraction force in sarcomeres.

The use of anti-CD68 antibody allowed us to observe the presence of a high number of macrophages, hetero-

geneously distributed in the conjunctive matrix of the myocardium in alcohol consumers, diagnosed with DCM (Figure 4c).

Through the immunomarking with anti-CD3 and anti-CD20 antibodies, we observed the presence of lymphocytes in the myocardial interstitium in patients with DCM (Figure 4d). In contrast, the immunomarking with anti-CD34 antibody showed that in DCM there is quite a developed microvascular network (Figure 4e), similar to that of a normal myocardium, which excludes the ischemic factor from the physiopathology of myocardial lesions.

The diagnosis of myocardial fibrosis was established in the patients where the changes of the cardiac extracellular matrix were characterized by an excessive diffuse accumulation of collagen fibers in interstitial and perivascular spaces. As observed from our images (Figure 5), myocardial fibrosis appeared focal or diffuse, mainly developed in the interstitial spaces around the blood vessels. It was accompanied by a multiplication of fibroblast conjunctive cells, simultaneously with the reduction of the number of myocardial muscular fibers (Figure 5a), the hypertrophy of remaining myocardial muscular cells (Figure 5b), or intense vascular congestion and stasis (Figure 5c).

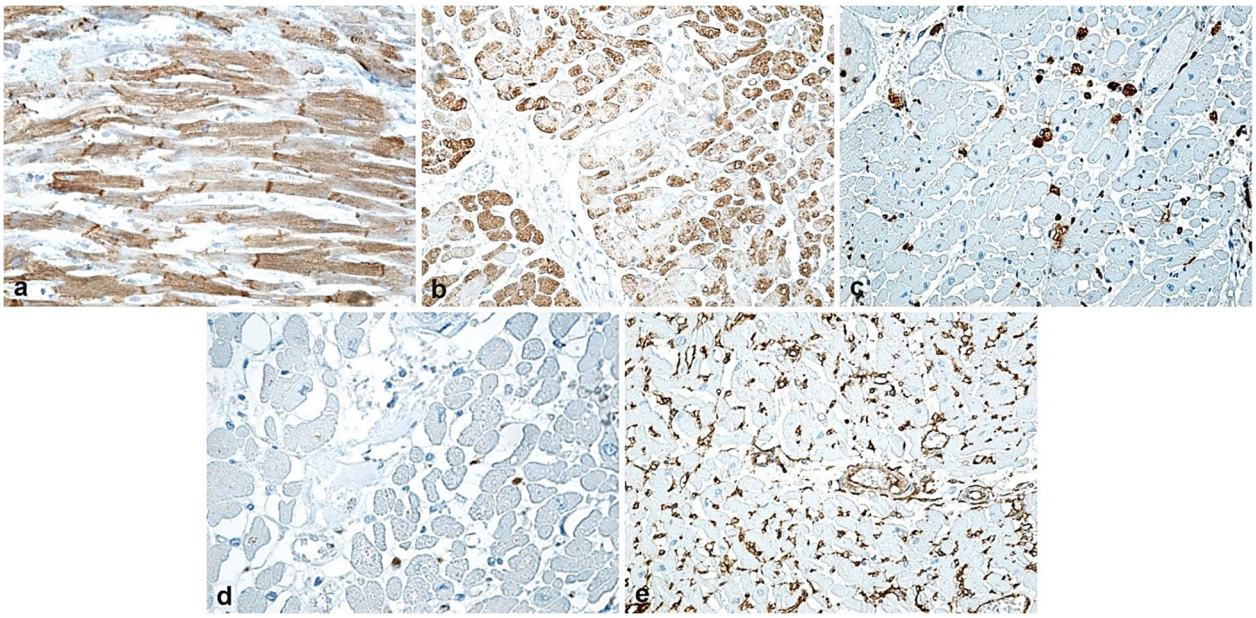

Figure 4 – ***Immunohistochemical aspects of the myocardium in subjects diagnosed with DCM: (a) Myocardial muscular fibers in longitudinal sectioning from an Ethanol consumer, diagnosed with DCM, where we may observe a heterogeneous reaction to anti-desmin, because of the reduction of desmin quantity in the sarcomere structure; (b) Transversal sectioning through myocardial fibers – we may observe quite a variable content of desmin in muscular fibers; (c) Numerous macrophages heterogeneously distributed in the myocardial fibers; (d) Rare CD3-positive T-lymphocytes in the myocardium interstitium, in patients with DCM; (e) Area of myocardium with a normal vascular density. Immunomarking with anti-desmin antibody: (a and b) ×200. Immunomarking with anti-CD68 antibody: (c) ×200. Immunomarking with anti-CD3 antibody: (d) ×200. Immunomarking with anti-CD34 antibody: (e) ×100. CD: Cluster of differentiation; DCM: Dilated cardiomyopathy.***

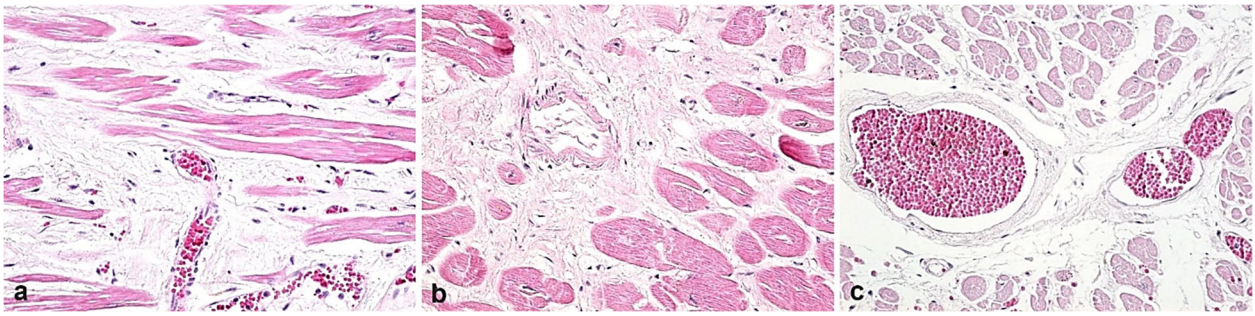

Figure 5 – ***Microscopic images from subjects diagnosed with DCM: (a) Diffuse myocardial fibrosis with the reduction of the number of myocardial fibers and the increase of conjunctive cell number in interstitial spaces; (b) Myocardial fibrosis mainly with an interstitial and perivascular development, associated with remaining muscular fibers hypertrophy; (c) Image of myocardial fibrosis with an interstitial and perivascular development, associated with congestion and vascular stasis. HE staining: (a–c) ×200. DCM: Dilated cardiomyopathy; HE: Hematoxylin–Eosin.***

The diagnosis of myocardial infarction was made based on the macroscopic and microscopic aspect of some areas in the left ventricle. We identified areas of late myocardial infarction, characterized by the presence of more or less extended fibrotic scars, with abundant collagen fibers, with few conjunctive cells and rare small blood vessels. At the periphery of the scar structure, there were highlighted rare muscular fibers of various sizes, with a heterogenous sarcoplasm (Figure 6, a and b).

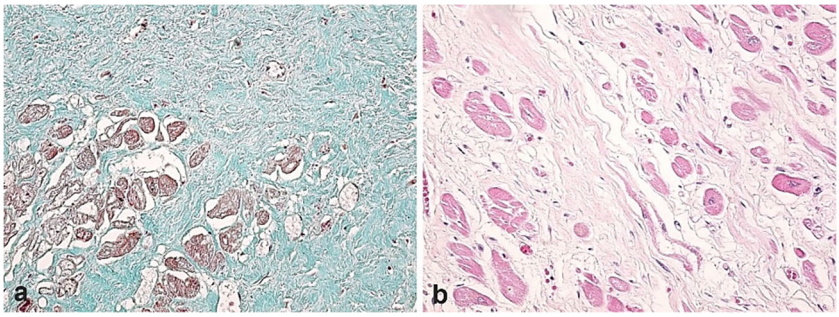

Figure 6 – ***Images of late myocardial infarction: (a) Area of dense, fibrous, extended conjunctive tissue, made of collagen fibers with a scattered arrangement, with few conjunctive cells and with rare small blood vessels (GS trichrome staining, ×200); (b) Dense fibrous scar replacing the loss of myocytes (HE staining, ×200).***

Discussions

Alcohol intake is a widespread habit around the world, with historical data showing that this drug has been used by people for over 7000 years [22]. Moreover, numerous studies, right from the end of the 20th century and the beginning of the 21st century, showed that if alcohol is consumed on a daily basis in low to moderate amounts, it has beneficial effects on the cardiovascular system, even in patients with ischemic heart disease [22–24]. However, the damaging effects on the heart of alcohol intake in large quantities began to appear in the literature as early as the 19th century [3]. It is now well-established that excessive alcohol intake has a negative effect on health and increases mortality [25, 26]. Some studies showed that alcoholism or chronic alcoholism is a pathological condition that occurs because of excessive alcohol intake, characterized by a strong desire, often uncontrollable, to consume alcohol. In Western countries, it is estimated that up to 10% of the adult population suffers from alcoholism [27]. Other studies show that, worldwide, alcohol is responsible for 5.3% of all deaths [28]. In the United States (USA) there are about 75 000 deaths each year due to excessive alcohol consumption [29, 30], and worldwide there are about 2.5 million deaths every year [31]. For some countries around the world, alcoholism has become a public health problem through large funds allocated to the care of patients who consume excessive amounts of alcoholic beverages. A study conducted in the USA estimated that during 2002, the economic cost of alcohol consumption was between 210 and 665 billion dollars [30, 32].

Consumed in excessive amounts, alcohol becomes a toxic drug with pathological effects on the liver, gastro-intestinal tract, cardiovascular system, kidneys, brain, etc. Practically, alcohol, due to its very small molecule, penetrates almost all tissues of the body, which leads to significant changes in organ function and the appearance of diseases limited to one organ or multisystemic disorders [33–36].

Our study was performed on a number of 77 individuals from the Nort-Eastern part of Romania, who died at home under suspicious circumstances and required a forensic examination at the IFM Iaşi. The autopsies were performed based on the ordinances of the Police Service of Iaşi to establish the cause of death. We make these clarifications, because alcohol consumption differs from one geographical area to another, even in the same country, being influenced by social, demographic, environmental, geographical, cultural, political, economic, religious factors etc.

In our study, it was noted that a fairly high percentage of individuals (40.3%), chronic alcohol users, were aged under 60 years old and came mostly from rural areas. International studies show that, in some countries, alcohol consumption begins in adolescence and increases in incidence after the age of 24 [37, 38]. Due to the high social, medical, and economic costs of alcoholism worldwide, the *World Health Organization* (WHO) has comprehensive strategies to reduce alcohol consumption [28, 39].

The lesions identified by us at the level of the heart, during autopsy, together with the HP and IHC examinations, were represented by DCM, myocardial fibrosis and myocardial infarction, injuries that we believe were caused by excessive alcohol consumption. Myocardial fibrosis was identified as an isolated lesion but was often identified in association with DCM or myocardial infarction.

In all patients, we determined the presence of EtG in the blood, which is a specific marker of recent alcohol consumption. As can be seen from our graphs, the values of this metabolite were elevated in all subjects, which, associated with heart changes, confirms chronic alcohol consumption. EtG is formed in the body by metabolizing ingested alcohol, being a stable, water-soluble, non-volatile metabolite that can be detected for a long time (80–96 hours) after the alcohol is eliminated from the body [40–43]. It is a reliable biochemical marker, being widely used in forensic medicine to highlight chronic alcohol intake. Since it can also be detected in urine [44] or hair [45], the determination of EtG can also be performed to monitor abstinence or alcohol consumption in other medical services (internal medicine, hepatology, psychiatry, etc.).

Compared to other known enzyme markers, EtG analysis exhibits greater sensitivity and specificity. Measuring the quantity of EtG in blood samples taken from bodies is the main evidence for chronic alcohol abuse.

The detection of the level of EtG in the blood, in parallel with the investigation data and the diagnosis following the necropsy, can lead to the diagnosis of chronic intoxication with Ethanol and the elucidation of inconclusive situations [46].

Based on the cases analyzed in the forensic laboratories, pathological anatomy, and forensic toxicology, in the cases of patients with known chronic alcohol abuse investigation, data showed significant HP changes in the heart, associated with a significantly elevated level of EtG in the blood [8, 9].

Regarding myocardial lesions, most of them were identified in cases with DCM. They were present both in the myocytes and in the myocardial intercellular conjunctival matrix. We believe that all injuries and myocardial changes are the results of alcohol abuse.

According to some studies, the cardiovascular system represents, after the liver and gastrointestinal ones, the second most affected by the global toxicity of Ethanol [47,

48]. Alcohol abuse can cause systemic atherosclerosis [49], high blood pressure (HBP) [50] and cardiac arrhythmias. However, in a very high percentage, it leads to progressive myocardial damage, also known as "alcohol dilated heart disease" [14, 24, 30, 51–53].

Chronic alcohol abuse represents one of the leading causes of DCM, characterized by dilated left ventricle and reduced systolic/diastolic function, ultimately progressing to congestive heart failure. Chronic alcohol exposure aggravates cardiac dysfunction because of volume overload. The cardiac response to alcohol reflects individual physiology, rate of consumption, and the amount of alcohol ingested. People with left ventricular dysfunction present the inhibitory effects of alcohol predominately, causing a decrease in the ejection fraction [54].

The HP aspects observed in the left ventricle myocardium of the subjects with DCM come to explain the macroscopic aspects of the heart identified at necropsy and to provide a morphological response to the clinical signs presented by patients before death. We believe that alcohol intoxication caused changes in myocytes consisting in the change of the structure of fibrillar and non-fibrillar proteins and also in intracellular organelles, which led to a different coloration of myocytes, with a decrease until the disappearance of the characteristic transverse striation. These findings denote a change in sarcomeres, accumulation of intracytoplasmic vacuoles, apoptosis, and necrosis of myocytes. The IHC study using the anti-desmin antibody showed a reduction of expression of this protein in some myocytes and, of course, damage to the structure of sarcomeres, with repercussions on myocardial contractility. Also, by using the anti-CD68 antibody, we highlighted the presence of many macrophages in the ventricular myocardium, cells that have the property to phagocyte cell debris resulting from myocyte death. The intercellular conjunctival matrix appeared more abundant, with a content of fibroblastic cells, which appeared as a restoring process of the myocardium after the destruction of myocytes, macrophages, collagen fibers, edema, and cell debris.

It is obvious that in the elderly, with other pre-existing lesions or malformations of the cardiovascular system or with other comorbidities (diabetes, hepatitis), the toxic effect of alcohol can be amplified [55–58].

Myocardial damage, in the case of alcohol abuse, is caused by both alcohol and the resulting metabolites, especially Acetaldehyde, by decreasing structural proteins and reducing myocyte contractility [59, 60].

The lesions produced in myocytes by alcohol are extremely varied (cell membrane, receptors, mitochondria, ribosomes, or cytoskeleton) because it has a highly reactive molecule, small in size and with a high diffusion capacity [61, 62]. Ethanol increases the permeability of the cell membrane, including intracellular organelles, disrupts the activity of ion channels (Ca^{2+}, Na^{+}/K^{+}) along with cellular signaling mechanisms and activates apoptosis [63, 64].

Other studies showed that alcohol disrupts the synthesis of ribosomal proteins, contributing to the depletion of non-contractile proteins in the sarcoplasm [62]. Excessive alcohol consumption causes the enlargement of the mitochondria with the appearance of megamitochondria, the degeneration of the folds of the inner membrane and even their fragmentation [65]. Mitochondria, in chronic alcohol intoxication, produce reactive oxygen species (ROS) that have either adaptive or harmful effects. Elevated ROS levels can eventually lead to apoptosis by promoting the oxidation of lipids, proteins, and deoxyribonucleic acid (DNA), as in alcohol DCM [66–68].

Our study confirms the observations of other authors who claim that chronic alcohol abuse reduces structural protein synthesis, with sarcomere disorganization, focal dissolution of myofibrils, cell vacuolation, and myocytolysis [69].

As we illustrated before, many studies show that changes in the intercellular conjunctival matrix are present in almost all cases of DCM caused by excessive alcohol consumption [70, 71]. The clearest one is myocardial fibrosis, which results from a process of tissue regeneration after apoptosis or myocyte necrosis. This myocardial regeneration is inefficient, the process of fibrosis being increasingly extensive, leading to the occurrence of left ventricular dysfunction and heart failure [72–74].

Conclusions

The examination of the 77 bodies in the IFM Iaşi, deceased at home under suspect circumstances, showed that chronic alcoholism may occur from teenage period until very old ages. Most chronic alcohol consumers were represented by men from the rural area, aged over 60 years old.

The macroscopic examination concluded that 62 (80.5%) subjects presented DCM, 71 (92.2%) subjects presented myocardial fibrosis, while nine (8.2%) individuals presented myocardial infarction.

In all the individuals included in the study group, there were determined high levels of blood EtG, thus showing the alcohol intake up to 96 hours before death.

HP examination of the subjects with DCM highlighted significant changes of myocytes in the left ventricle myocardium, consisting in changes of the sarcoplasm, of myofibrils, myolysis, apoptosis and necrosis of cardiac muscular fibers. The IHC studies showed a low content, variable from one myocyte to another, of desmin and the presence of a high number of macrophages in the myocardial interstitium.

The inflammatory reaction was absent, T- and B-lymphocytes being identified in a very low number. The anti-CD34 antibody immunomarking showed that, in DCM, there is a well-developed microvascular network similar to that of a normal myocardium, thus proving the fact that Ethanol DCM has a non-ischemic feature.

Myocardial fibrosis appeared focal or diffuse, mainly developed in the interstitial spaces around blood vessels, being accompanied by a multiplication of fibroblast conjunctive cells, simultaneously with the reduction of the myocytes number.

Conflict of interests

The authors declare that they have no conflict of interests.

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