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*Asociația de Biosiguranță și Biosecuritate din Republica Moldova (ABBRM)* este o organizație profesională cu caracter științifico-practic și instructiv-educativ, neguvernamentală, apolitică și nonprofit, creată în 2017.

Obiectivul principal al asociației este dezvoltarea bunelor practici și culturii în domeniul biosiguranței și biosecurității și promovarea cunoștințelor în cadrul grupurilor profesionale și de cercetare-inovare.

**Biosiguranța** – include principii de securizare, tehnologii și reguli ce trebuie urmate pentru a preveni expunerea neintenționată la agenți patogeni și toxine sau eliberarea/scurgerea lor accidentală.

„Protejarea personalului, populației de expunerea neintenționată la patogeni/material cu biohazard”.

**Biosecuritatea** – include un spectru larg de măsuri (politici de biosecuritate, regim de reglementări, măsuri științifice și tehnice) aplicate într-un cadru organizat, necesar minimalizării riscurilor (prevenirea acțiunilor, atentatelor teroriste de eliberarea intenționată de patogeni sau toxine precum și a pierderii, furtului sau folosirii greșite a acestora).

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**Managementul riscului** – este un proces de luare a deciziilor în urma cărui rezultate din evaluarea riscului (procesul de estimare a pericolelor la locul de muncă) sunt integrate cu principii economice, tehnice, sociale și politice pentru generarea unor strategii de reducere a riscului.

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## *One Health for better world and better public health*




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**MD, PhD, Professor of the Department of Public Health, Head of**  
**Quality Assurance Department, Faculty of Medicine, Ivane**  
**Javakhishvili Tbilisi State University, Georgia**

Public Health is the Science and Art of protecting and improving the health of people, promoting healthy lifestyles, preventing diseases and injuries and responding to infectious diseases.

In recent years it became more evident that effective public health interventions require a close cooperation between people, animals, plants, and the environment. A holistic, multisectoral, multidisciplinary, and integrated approach on protecting human health is needed at national as well as at regional and global levels.

One Health approach is crucial not only for preventing vector-borne and zoonotic diseases, but also for non-communicable diseases and injury prevention. The concept One Health covers issues related to food safety and food security, environmental contamination, and other threats. Even occupational health can benefit from „One Health”.

The Journal „One Health&Risk Management” funded in 2019 by Moldavian Biosafety and Biosecurity Association (MDBBA) disseminates important and valuable results of scientific researches from different disciplines under the umbrella „One Health” which promotes a better world and better public health.



**SYNTHESIS ARTICLE – ARTICOLE DE SINTEZĂ –  
ARTICLES DE SYNTHÈSE – ОБЗОРНЫЕ СТАТЬИ**



**ENDOTHELIAL DYSFUNCTION IN NONALCOHOLIC FATTY LIVER DISEASE**

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**Keywords:** endothelial dysfunction, nonalcoholic fatty liver disease, nitric oxide.

**Introduction.** The prevalence of nonalcoholic fatty liver disease (NAFLD) in western countries is increasing rapidly and is considered as component of metabolic syndrome. Endothelial dysfunction is a pathophysiological problem of cardiovascular disease. NAFLD, as a component of metabolic syndrome, is associated with endothelial dysfunction.

**Material and methods.** PubMed database was used in order to review and select articles according to the keywords. A total of 216 articles matching search criteria were found between 2000-2021.

**Results.** The present study has been underlined the role of pathophysiological mechanisms of endothelial dysfunction in nonalcoholic fatty liver disease, that involves oxidative stress, inflammation and insulin resistance. The main factor that influences the occurrence of endothelial dysfunction is related with nitric oxide (NO) biosynthesis. The markers which associated with regulation of nitric oxide biosynthesis, such as asymmetric dimethylarginine, free fatty acid, lectin-like oxidized low density lipoprotein (LDL) receptor-1 and pentraxin-3, are potential targets in assessment of endothelial dysfunction.

**Conclusions.** Insulin resistance, inflammation and oxidative stress have involved in reduction of NO biosynthesis that influence occurrence of endothelial dysfunction. Markers, such as lectin-like oxidized LDL receptor-1 and pentraxin-3, have considered as potential targets in assessment of endothelial dysfunctions in NAFLD.

**Cuvinte cheie:** disfuncție endotelială, boala ficatului gras non-alcoolic, oxid nitric.

**DISFUNȚIA ENDOTELIALĂ ÎN BOALA FICATULUI GRAS NON-ALCOOLIC**

**Introducere.** Prevalența bolii ficatului gras non-alcoolic (BFGNA) în țările occidentale este în creștere rapidă și este considerată ca o componentă a sindromului metabolic. Disfuncția endotelială este o problemă fiziopatologică a bolilor cardiovasculare. BFGNA ca o componentă a sindromului metabolic este asociată cu disfuncția endotelială.

**Material și metode.** Baza de date PubMed a fost utilizată pentru a revizui și selecta articole în funcție de cuvintele cheie. Pentru perioada 2000-2021 au fost găsite 216 articole care au corespuns criteriilor de căutare.

**Rezultate.** Prezentul studiu a subliniat rolul mecanismelor fiziopatologice ale disfuncției endoteliale în boala ficatului gras non-alcoolic, care implică stresul oxidativ, inflamația și rezistența la insulină. Factorul principal care influențează apariția disfuncției endoteliale este legat de biosinteza oxidului nitric (ON). Markerii care sunt asociați cu reglarea biosintezei oxidului nitric, cum ar fi dimetilarginina asimetrică, acizii grași liberi, lectin-like oxidized low density lipoprotein (LDL) receptor-1 și pentraxin-3, sunt potențialele ținte pentru evaluarea disfuncției endoteliale.

**Concluzii.** Rezistența la insulină, inflamația și stresul oxidativ sunt implicați în reducerea biosintezei a ON, ce stă la baza apariției disfuncției endoteliale. Markerii, precum lectin-like oxidized LDL receptor-1 și pentraxin-3, sunt considerați ca ținte potențiale pentru evaluarea disfuncției endoteliale în BFGNA.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease with high prevalence in western world (1). The prevalence of NAFLD is higher than 25% (2). NAFLD is considered to be component of metabolic syndrome that is defined as combination of abnormalities that includes obesity, hypertension, dyslipidemia and hyperglycemia (3). NAFLD is a group of conditions occurring in patients without alcohol consumption, it has broad spectrum of manifestation ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), more severe form of NAFLD, which eventually progresses to cirrhosis and hepatocellular carcinoma (HCC) (4). Insulin resistance is a characteristic feature of NAFLD (5). Study shows that insulin resistance plays major role in imbalance of the nitric oxide (NO) dependent vasodilator and endothelin-1 (ET-1), which lead to endothelial dysfunction (ED) (6). The incidence of cardiovascular disease has increased significantly in patient with NAFLD (7). The relation between endothelial dysfunction and NAFLD among patient with absence of any risk factors for cardiac disease is established (8). This explains that NAFLD isn't related to comorbidity, but it might be involved in cardiovascular disease (CVD) pathogenesis. Liver releases some mediators such as C-reactive protein (CRP), fibrinogen and plasminogen which is considered pro-atherogenic, and can be related to the pathogenesis of CVD and endothelial dysfunction (9). Endothelial dysfunction is a predictable factor that increases risk of development of atherosclerosis (10). Assessment flow mediated dilatation (FMD) of brachial artery most common noninvasive technique for diagnosis of endothelial dysfunction (11). NAFLD is associated with endothelial dysfunction and arterial stiffness (12).

*The aim of this article is to analyze the role of endothelial dysfunction in development of nonalcoholic fatty liver disease and to examine the methods of assessment of endothelial dysfunction.*

## MATERIAL AND METHODS

We performed a systematic review to analyze the pathophysiology, markers and emerging therapy of endothelial dysfunction in NAFLD. We searched for articles on *PubMed* database by applying the following keywords: endothelial dysfunction, nonalcoholic fatty liver disease, nitric oxide. We selected 47 articles that we deemed

relevant to the proposed research topic out of a total of 216 articles matching the search criteria found between 2000-2021.

## RESULTS

### *Pathophysiology of endothelial dysfunction in NAFLD*

The term "endothelial dysfunction" typically is characterized by abnormalities in the production or bioavailability of endothelial-derived nitric oxide (NO), increase oxidative stress in endothelium, and eventually leads to abnormal pro-thrombotic, pro-inflammatory conditions, vasoconstriction and resultant changes in vascular reactivity (13). The endothelium is composed of monolayer cells, called endothelial cells that play major role in normal vascular wall function (14). Distribution of this layer is characterized by circulating endothelial progenitor cell (EPC) which plays the major role in regeneration of the endothelial lining of blood vessels. Level of endothelial progenitor cell in patient with NAFLD were decreased and their function were attenuated, which have correlated with endothelial dysfunction. The maintenance of endothelium wall is important in protecting against atherosclerosis (15). Endothelial dysfunction leads to imbalance in generating vasodilator substance (NO, endothelium-derived hyper-polarizing factor (EDHF) and prostacyclin) and vasoconstrictor substance (angiotensin II, secretory ET-1, norepinephrine, leukotriene and thromboxane A) essential substance for vascular homeostasis (16). When the irregularly production of vasoactive vasodilator substances occurs, this provokes the vasculature towards pro-thrombotic and pro-atherogenic effects (leukocyte adhesion, platelet activation, pro-oxidation, impaired coagulations, vascular inflammation, atherosclerosis and thrombosis) (17).

### *Insulin resistance*

The liver contain fat that seems to be the best predictor of insulin resistance in adipose tissue, skeletal muscle and liver. Insulin resistance at the level of endothelium can be detected before progression to inflammation, cirrhosis or any other sign of advanced NAFLD (18). Endothelial dysfunction has been related to insulin resistance that is an early common finding in patients with metabolic syndrome (19) and the main pathophysiological hallmark of NAFLD (20). The main factor in development NAFLD is the insulin resis-

tance which cause metabolic abnormalities that include glucotoxicity, lipotoxicity, and inflammation which also lead to endothelial dysfunction. In the presence of insulin resistance, insulin signaling system is disrupted, pathway-specific phosphoinositide 3-kinase dependent signaling is impaired and induce reduction in production of NO, leading to endothelial dysfunction (21). Insulin resistance increase the possibility of patient with ED to develop cardiovascular complications (atherosclerosis, diabetes, dyslipidemia, hypertension and coronary heart disease).

#### *Nitric oxide and endothelial dysfunction in NAFLD*

NO is an important protective molecule and main biochemical mediator of endothelium-dependent vasodilation in blood vessels (22). NO is produced by endothelial nitric oxide synthase (eNOS) in response to oxidative stress and vasoconstriction stimuli and has vasodilatory function in regulation of blood flow and blood pressure. Activation of eNOS suggest an increase of intracellular calcium ( $Ca^{2+}$ ) and binding of  $Ca^{2+}$ /calmodulin to the enzyme. This pathway can be stimulated by oxidative stress and insulin resistance and lead to decrease in NO production and provoke endothelial dysfunction (23). Inflammation and oxidative stress are important factors that influence appearance of endothelial dysfunction and NO bioavailability reduction, which is important in vascular homeostasis. Reduced NO bioavailability (due to decrease NO production or NO breakdown induce by the chemical reaction with oxidant radicals) can same lead to endothelial dysfunction (fig. 1).

#### *Oxidative stress and endothelial dysfunction in NAFLD*

Oxidative stress is provoked by overproduction of reactive oxygen species (ROS) in the cells and tissue. Overproduction of ROS can cause tissue imbalance, cell injury (24) and lead to ED. Inflammation plays major role in determination of endothelial dysfunction caused by ROS overproduction (25). The overproduction of ROS occurs with the reduction of NO and nitric oxide synthase (NOS) level. NO react with superoxide anion  $O_2^-$  to produce most powerful oxidant peroxynitrite (ONOO<sup>-</sup>), which generates vasoconstriction, decreases the bioavailability of NO and influences the vasodilator response. Together, the reduction in NO synthesis and the uncoupling of eNOS lead to the loss of vascular tone regulation, especially the NO-dependent vasodilatation producing ED.

The vascular hypertension is favored by ED, leading to worsening of the portal hypertension prognosis and contributes to the development of new vascular events, such as atherosclerosis (26, 27). ED in early stage of NAFLD is related to a decrease in NO bioavailability combined with elevated end product of cyclooxygenase and oxidative stress. Both pathways are involved in pathophysiology and may help to develop the treatment goals to stop disease evolution (28).

#### *Inflammation and endothelial dysfunction in NAFLD*

Oxidative stress cause release of inflammatory cytokines that play major role in development of endothelial dysfunction (29). Liver release C-reactive protein, fibrinogen and plasminogen which is considered pro-atherogenic. There is a strong association between insulin resistance that plays a major role in endothelial dysfunction, and C-reactive protein. The fibrinogen and plasminogen-1 activation inhibitor (PAI-1) also are released from liver and activate the coagulation system. *Targher et al.*, confirm that patient with NAFLD had higher levels of high sensitivity CRP, fibrinogen, and PAI-1 (9). The renin-angiotensin system (RAS) has an important role in regulating vascular function. Angiotensin-2 is the main component of RAS. The effect of angiotensin-2 on endothelial dysfunction is regulated by interaction with the plasma receptor membrane angiotensin-2 type 1 and leads to NO reduction by inducing eNOS and promoting NOS uncoupling (30). Nuclear factor kappa-B (NF-Kb) is a transcription factor that plays a major role in intrahepatic inflammation and oxidative stress. Increasing level of NF-Kb lead to hepatic production of inflammatory cytokines interleukin-6, interleukin-1b and tumor necrosis factor alfa (29).

#### *Markers of endothelial dysfunction in NAFLD*

ED assessment is one of the most recent research areas in the field of NAFLD, and its evaluation may be essential to define patients with a higher risk of developing of cardiovascular diseases. A possible joining link between NAFLD and cardiovascular diseases has therefore been identified in ED. For this reason, in order to predict the cardiovascular risk of NAFLD patients, it is necessary to develop new diagnostic methods that can measure ED.

Therefore, for ED evaluation it is possible to use invasive methods (intravascular injection of acetylcholine and the measurement of vasodilation



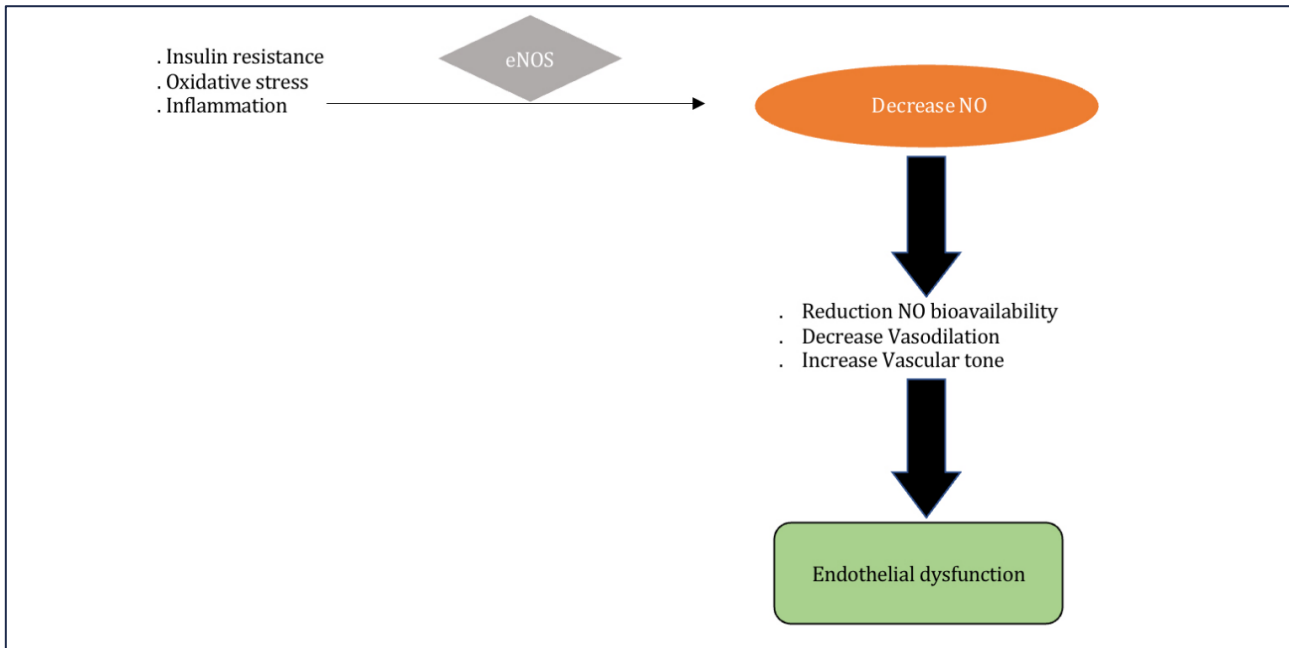


Figure 1. Pathophysiology of endothelial dysfunction in nonalcoholic fatty liver disease.

caused by this neurotransmitter). Economically unfavorable non-invasive methods for screening ED dysfunction (flow mediated dilatation FMD), up to dosage of ED serum markers. However, we focus on non-invasive, inexpensive, and useful biomarkers in clinical practice. Hence, there is a need to study the role of circulating biomarkers in relation to endothelial dysfunction and the severity of the underlying liver disease.

*Asymmetric dimethylarginine*

The methylated arginine is a natural occurring product of metabolism regulated by a hepatic enzyme called dimethylarginine diaminohydrolase (DDAH). There is two isoforms of DDAH exist in human, DDHA-1 it's isoform that participate in regulation of hepatic and systemic asymmetric dimethylarginine (ADMA) and exists in the expressing neuronal nitric oxide synthase (nNOS). The second form is DDAH-2 that has important function in regulating NO activity, and present in tissue that expressing eNOS. Therefore, increased intracellular DDAH has an important role in regulating ADMA. Dysfunction of DDAH activity can lead to increasing of intracellular ADMA concentration and reduction in NO signaling, which induce endothelial dysfunction (31). The overexpression of DDAH-1 in human endothelial cells shows a moderate increase in NO concentration by 3 times (32). DDAH1 is one of the target genes of farnesoid X receptor (FXR). Treatment of cirrhotic rats with FXR agonists can restore NO

levels (33). Colak Y. et al. (34) suggested that the plasma levels of ADMA were higher in patients with NASH, and there was no significant difference between any NAFLD patients' group and control group. Therefore, it can be suggested that possible treatments for diseases or endothelial dysfunction may effectively reduce the cardiovascular risk of NAFLD patients.

*Free fatty acid*

The liver plays a key role in lipid homeostasis, regulation of transport and lipid synthesis, abnormal lipid profile it can be associated with development of liver disease. Elevated free fatty acid (FFA) in blood is considered as an important link between insulin resistance, inflammation, obesity, type 2 Diabetes Mellitus (T2DM) and hypertension (HTN). Dyslipidemia, which is frequently associated with NAFLD, increase risk for endothelial dysfunction (35). Insulin resistance, oxidative stress, and inflammatory burden are important causes of FFA-induced ED (36). Free fatty acid-mediated endothelial dysfunction includes many mechanisms that involves impaired of the insulin receptor substrate/phosphatidylinositol 3 kinase pathway of insulin signaling and nitric oxide production. Oxidative stress and inflammation (through activation nuclear factor-kappa B) lead to release pro-inflammatory, pro-atherogenic cytokines that activate the renin-angiotensin system and apoptosis in the endothelial cells. Moreover, the increase in free fatty acid levels caused

by metabolic syndrome is considered to be an important link in the occurrence of endothelial dysfunction (37). Therefore, previously provided information demonstrates that FFA can be a predictable novel biomarker for ED in NAFLD.

#### *Lectin-like oxidized LDL receptor-1*

It has been identified as the key receptor for oxidized low-density lipoprotein in endothelial cells, and regarded as a marker for ED in assessing pathological condition such as atherosclerosis (38). Lectin-like oxidized LDL receptor-1 (LOX-1) promote ROS generation, augments endothelial adhesion to monocytes and inhibit NO synthesis (39). Study, represents that serum LOX-1 increased in patients with NAFLD compared to healthy individuals (40) and LOX-1 may be one of the marker for endothelial dysfunction in NAFLD.

#### *Pentraxin-3*

Pentraxin-3 (PTX-3) is a prototype protein that belongs to a pentraxin family. Elevated level of PTX-3 is reportedly associated with obesity, metabolic syndrome and cardiovascular disease. PTX3 is involved in endothelial dysfunction by various mechanisms, decreases the synthesis of NO, inhibits cell proliferation and alters its functions. Elevated PTX-3 is highly associated with endothelial dysfunction in NAFLD and may present interest as a marker for ED in NAFLD (41, 42).

#### ***Emerging therapy of endothelial dysfunction in NAFLD***

Endothelial dysfunction has associated in the pathogenesis of NAFLD. It seems that restoring ED is a very important therapeutic goal in NAFLD

management. NAFLD pharmacotherapy hasn't yet been determined. The only treatment that is proved is non-pharmaceutical treatment that includes lifestyle changes, weight loss, physical exercises and proper diet, are the only treatment recommendations that shows proven benefits (43). Novel pharmacotherapy of ED in NAFLD strategy based on underlying disease related factors as the disease progresses (oxidative stress, inflammation, FFA and insulin resistance). Statins which have anti-inflammatory and antioxidant effects, due to cholesterol lowering effect, improve endothelial function reduce hepatic lipid content and serum alanine aminotransferase (44). There is a study demonstrating the association of endothelial dysfunction with angiotensin converting enzyme (ACE) inhibitors, suppresses the degradation of bradykinin and stimulates the bradykinin receptor of the endothelial cell to produce NO and has an important role in preventing the development of endothelial dysfunction (45). The study shows that combination of both statins and ACE inhibitors results in improving function of endothelium and promote amelioration of inflammation (46). It's recommended that patients with ED in NAFLD to undergo medical analysis of liver enzyme before prescribing any medication, instead of detecting an increase in liver enzymes due to the usage of prescribed medication. This process it must be indicated in all type of drugs that has beneficial effects in treatment of endothelial dysfunction (ACE inhibitors, calcium antagonist, beta blockers, statins, insulin resistance improving drugs, renin blockers and antioxidants) (47).

## **CONCLUSIONS**

1. Insulin resistance, inflammation and oxidative stress are involved in reduction of nitric oxide biosynthesis that influences the appearance of endothelial dysfunction. Therefore, markers such as lectin-like oxidized low density lipoprotein receptor-1 and pentraxin-3 are considered as potential target in assessment of ED in NAFLD. Furthermore, NO regulator like dimethylarginine diaminohydrolase could be considered as possible target for therapeutic management. Treating the ED in NAFLD with NO modulators might suppress disease progression. However, further research must be carried out to understand ED markers and the importance of their effect in the assessment of NAFLD.

## **CONFLICT OF INTERESTS**

No conflict of interests.

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## GOLD NANOPARTICLES FROM MAGNETITE FOR THE DETECTION OF AMYLOID PROTEINS IN NEURODEGENERATIVE DISEASES

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**Keywords:** nanoparticles, gold, magnetite, amyloid proteins, neurodegenerative diseases.

**Introduction.** Currently, neurodegenerative diseases (ND) are the fourth leading cause of death worldwide that pose a great challenge in the development of tools for early diagnosis. Thus, advances in science seek sensitive and selective detection systems and this manuscript will highlight the importance of nanotechnology.

**Material and methods.** A literature review was conducted on the representative findings of NPs technologies in neurodegenerative diseases. Articles written in both English and Spanish were included. References between 2015-2021 were also taken into account.

**Results.** One of the most representative techniques, AuNP was specifically implemented, together with a magnetic center composed of magnetite, which has as a specific ligand with a C-terminal cysteine domain present in the B-amyloid protein, which adhere directly to the surface of the NPs, characterizing the anomalous protein. Subsequently, by means of nanosensors capable of detecting and measuring different concentrations, these pathologies are identified at an early stage.

**Conclusions.** Today, along with the advent of biotechnology, it has been possible to design techniques with NPs that allow the identification of specific mutations and provide diagnosis in individuals. In the investigative models of AuNP, it is possible to infer that the capabilities that make them representative focus on their magnetism and biofunctionality, by specifically binding to amyloid peptides and other ligands present in the protein, which are the major components of amyloid plaques used in these studies.

**Cuvinte cheie:** nanoparticule, aur, magnetită, proteine amiloide, boli neurodegenerative.

### NANOPARTICULE DE AUR DIN MAGNETIT PENTRU DETECȚIA PROTEINELOR AMILOIDE ÎN BOLILE NEURODEGENERATIVE

**Introducere.** În prezent, bolile neurodegenerative (BN) constituie a patra cauză de deces la nivel mondial, reprezentând o mare provocare în dezvoltarea instrumentelor de diagnosticare precoce. În acest sens, se fac cercetări științifice care au în vizor sisteme de detecție sensibile și selective, de aceea în acest rezumat va fi relevată importanța nanotehnologiilor.

**Material și metode.** A fost efectuată o cercetare a literaturii de specialitate cu privire la constatările reprezentative ale tehnologiilor nanoparticulelor (NPs) în bolile neurodegenerative. Au fost incluse articole scrise atât în engleză, cât și în spaniolă. Au fost luate în considerare și referințe apărute în 2015-2021.

**Rezultate.** Într-una dintre cele mai reprezentative tehnici, au fost implementate în mod specific NPAu (nanoparticule de aur), împreună cu un centru magnetic compus din magnetit, care are ca ligand specific un domeniu cisteinic C-terminal, prezent în proteina B-amiloid, acestea aderând direct la suprafața nanoparticulelor, care caracterizează proteina anormală. Ulterior, prin intermediul nanosenzorilor, capabili să detecteze și să măsoare diferite concentrații, aceste patologii sunt identificate într-un stadiu incipient.

**Concluzii.** Odată cu apariția biotehnologiei, a fost posibilă se proiecteze tehnici ce utilizează NP, care permit, la ora actuală, identificarea mutațiilor specifice și diagnosticul indivizilor. Grație modelelor de investigație cu NPAu, putem deduce că capacitățile, care le fac reprezentative, se concentrează pe magnetismul și pe biofuncționalitatea lor, prin legarea specifică de peptide amiloide și de alți liganzi prezenți în proteină, componente majore ale plăcilor de amiloid utilizate în aceste studii.

## INTRODUCTION

Currently, nanotechnology has taken a broad interest in different branches of biomedicine. In order to solve problems with potential risk to health, it is vital that different disciplines work together, thus strengthening concepts, themes and processes, which together propose assertive solutions in order to achieve optimal quality for the system called Planet Earth.

The US Environmental Protection Agency (EPA) classified particles into three general categories with respect to their size, starting with a defined range between 10,000 and 2,500 nanometers (nm) for those particles called coarse, followed by fine particles ranging between 2,500 and 100 nm, and the last classification included the category ultrafine or nanoparticles (NPs) ranging between 100 and 1 nm. It should be noted that these nanostructures can be generated from various materials, from metal to ceramic composites. Regarding the above mentioned, the NPs that have adopted a greater interest and detailed research in the forefront, are those that in their composition have magnetic elements formed by iron oxide, since they have biofunctional physical properties. Among its most important characteristics is its magnetic core accompanied by a polymeric shell that has the ability to bind molecules to its surface, as well as the high ratio between the surface area/size, in addition to its biocompatibility and easy biodegradation in the body (1).

The term "nano" is used to describe scientific areas and technologies that work with materials that possess at least a dimension of less than 100 nm, that is, the construction of structures at the nanometric scale with unique properties through the manipulation of atoms and molecules, being called nanomaterials. Therefore, they can represent an optimal model as biomarkers for an early clinical diagnosis of malformed proteins or amyloid proteins present in neurodegenerative diseases (ND), since these pathologies are characterized by prolonged incubation periods, associated with a slow and irreversible fatal evolution, thus, preventing the spread of these deposits in the brain, which are the earliest key events in the progression of diseases and delaying this deterioration of poor prognosis (2).

In addition, there may occur various behavioral changes, hypersensitivity, tremors, intense itch-

ing, ataxia, excitability, and seizures; vacuolization, astrogliosis and neuronal death may develop in the brain. Once the abnormal prion proteins appear, they bind and form fibers or accumulations in the central nervous system, called amyloid plaques, which may start accumulating years before the symptom onset. (3).

The prion protein, in its normal version (PrP<sup>c</sup>), after being synthesized, is modified in the Golgi apparatus and then transported to the cell surface; it is present in mammalian neuronal membranes. Recent studies have shown that it is involved in synaptic transmission, signal transduction, antioxidant activity of superoxide dismutase, neuroplasticity and cell survival. These neurodegenerative conditions can develop because a person's normal prions spontaneously change to the infectious form of the protein (PrP<sup>Sc</sup>) and then alter prions in other cells in a chain reaction. The difference between the two isoforms is that PrP<sup>c</sup> has 40%  $\alpha$ -helices and less than 10%  $\beta$ -sheets in its tertiary structure, whereas PrP<sup>Sc</sup> has about 50%  $\beta$ -sheets, which makes it insoluble in non-denaturing detergents, partially resistant to proteinase K and highly resistant to sterilization processes, as well as to physical and chemical agents capable of degrading viral nucleic acids. The amino acid sequence of PrP<sup>c</sup> and PrP<sup>Sc</sup> can be referred to as isoforms (4).

Thus, amyloidoses represent a spectrum of diseases resulting from the pathological deposition of fibrils of about 28 different protein molecules including immunoglobulin light chains, polypeptide hormones, transport molecules, transthyretin, amyloid A polypeptide, Tau protein, amyloid precursor protein, huntintin and others (5).

Therefore, for the use of these NPs as biomarkers of amyloid proteins it is of vital importance to determine that those new metals with magnetic core components to be used lack cytotoxic effects and are absolutely biocompatible; therefore, magnetite is one of the research focuses. After being metabolized, the iron ions of these particles are added to the iron deposits of the organism and eventually incorporated by the erythrocytes as part of the hemoglobin. In addition, they have a gold coating that protects the iron oxide nuclei from oxidation when present in body fluids, providing optical properties and a surface with a good capacity to be biofunctionalized (6).

Magnetite ( $\text{Fe}_3\text{O}_4$ ) is found disseminated as an accessory mineral in many igneous rocks sometimes forming large masses of ore that are generally very titaniferous and appears associated with crystalline metamorphic rocks. In mining, gold is separated from magnetite, after the separation, synthesis methods are used to obtain gold nanostructures. In Colombia magnetite is totally wasted, important and considerable amounts of magnetite can be found at the end of the processes of the deposits, reaching even more than 40% in weight of the material treated to obtain gold. This percentage of magnetite is totally wasted after the fire assays that are carried out in an artisanal way, aggravating the environmental problem and favoring the continuity of mineral waste (7).

### MATERIAL AND METHODS

A literature review was conducted on the representative findings and diagnostic potential of AuNP technologies in neurodegenerative diseases. This review was carried out from a scientific database, using ScienceDirect, PubMed, Scopus, Web of Science, during the month of March of this year.

Articles written in both English and Spanish were included, a search that yielded 50 articles, of which 20 were filtered that met the requirements of the study in progress. References between 2015-2021 were also considered as related publications of interest.

The focus of the manuscript is concurrent to the ENs that are currently the fourth leading cause of death worldwide, advances in science seek to make an early diagnosis to the development of these. One of the most representative methods consists in the design of a supercrystal capable of acting as a nanoantenna that identifies the molecule by means of a biofilm interpolated on the surface of the AuNP for the aggregation of a specific part in the peptide conformation of the protein of interest, then the supercrystal is immersed with an optical sensor in a sample of plasma or centrifuged blood. The sensor then generates an extremely high electric field on the crystal surface, quantifying its presence (8).

### RESULTS

The prion theory assumes the existence of two

foldings for a single amino acid sequence and the refolding of normal PrPc by the action of pathological PrPsc, suggesting a flow of information from one protein to another at the level of tertiary structure. For this reason, prions are the only living particles that contradict the central dogma of biology (9). In addition, some theories assure that PrPc has a viral origin, these affirm that the viral gene could belong to a retrovirus that infected a vertebrate from which the rest of them evolved. Therefore, the gene was incorporated into the genome forever. However, despite this, there is still no accepted study that fully shows what could be the origin of this protein (4).

It should be noted that there is currently no function of PrPc that has been specifically determined. Although some scholars mention the possibility, that PrPc protein has a possible role in copper metabolism and antioxidant defense. In addition, there is evidence that it would have enzymatic function of superoxide dismutase (SOD), as well as interaction with other proteins.

Likewise, PrPc in endothelial cells that form part of the blood-brain barrier accumulates at cell-cell junctions and participates in the transmigration of monocytes from peripheral tissues to the brain possibly by specific recognition of certain molecules on the surface of monocytes (10).

In some proposed methodologies, such as cultured cells treated with neuronal growth factor (NGF), it is mentioned that PrPc favors neuritogenesis through the laminin-PrPc complex, so it is thought that it could be involved in ligand recognition and cell adhesion, by which proliferation and survival signals are triggered in cells.

PrPc is able to bind to heparins and heparin-like compounds. Heparin is a sulfated polyanion similar to glycosaminoglycans, which are part of the composition of amyloid plaques that arise in the presence of PrPsc. Heparin molecules sequester PrPc preventing it from binding to glycosaminoglycans by competition. It also binds to NCAM protein, a neuronal adhesion molecule, NF-E2-related factor 2, which is a transcription factor, Bcl-2 and apolipoprotein E, a membrane protein involved in Alzheimer's disease. In addition, this occurs in the clathrin-coated holes of the plasma membrane.

Another protein characterized as a ligand of PrPc is the 37 kDa laminin receptor precursor. It was

detected that there is interaction of this receptor and PrPc both in vitro and in vivo and it is also overexpressed in organs that accumulate PrPc. It is currently believed that this is the receptor for PrPc in mammalian cells in vivo, although it is most likely not the only one.

Through its localization in the membrane, PrPc could participate in signal transduction path-

ways. According to some authors, prion infection affects the function of calcium channels.

Nanoparticles represent an appropriate tool for potential biomedical diagnostic and therapeutic applications due to their ability to be biofunctionalized and guided to a specific region of the organism by an external magnetic field (fig. 1) (1).

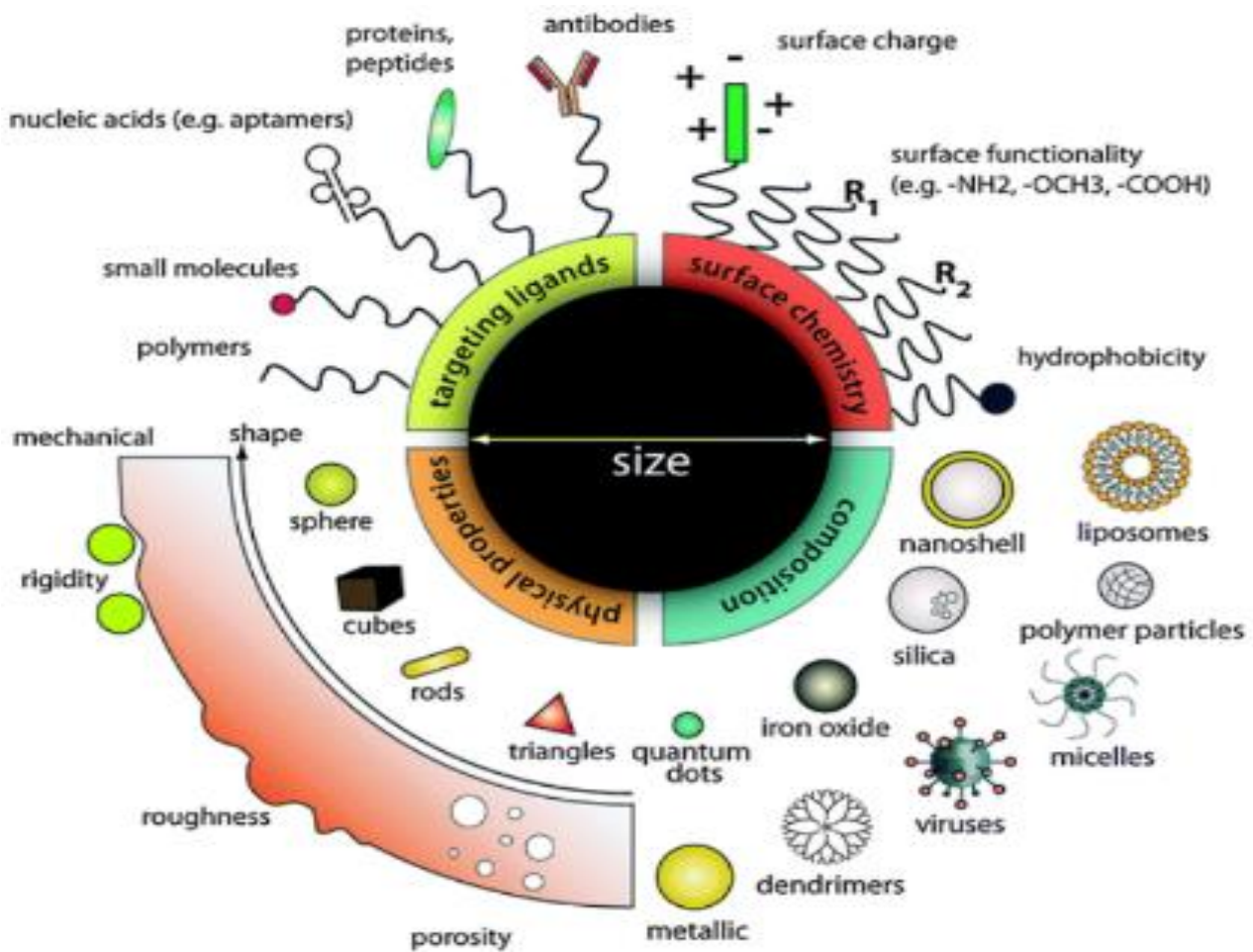


Figure 1. Nanoparticles. Characteristic aspects that make a nanoparticles a sensitive and selective material to be used as a diagnostic technique (1).

In the same way, since these nanomaterials have different usable characteristics and by means of different chemical reactions, they can be directed to different biological molecules with the final objective of being recognized, thanks to ligands expressed by molecules of interest, and thus, achieve their accumulation to be magnetically marked. Due to the limitation imposed by the low concentrations of PrPc in certain biological samples, they are not detectable using other

conventional methods, i.e., those would allow their aggregation.

The NPs are defined as a particles of matter that is between 1 and 100 nanometers in size. NPs emerged as tools for therapeutic, diagnostic and drug delivery applications. They can be synthesized from a wide range of materials, such as polymers, metals or carbon-based molecules. NPs are also highly functional due to the ease with which their shape, size and surface proper-



ties can be modified; they can also be altered by binding other substances to the surface or trapping them within their cavities (11).

Several tools based on nanobiosensors have been designed and tested as markers for the diagnosis of ND by implementing gold, since it is a very stable metal also at the nanoscale. It is a good conductor of electrons and has a strong response when excited by an optical field (12).

The microspheres signal and detect beta-amyloid in patients with NAFLD, where samples are collected from cerebrospinal fluid (CSF), serum and plasma for detection. They help to immobilize the altered protein, thus promoting efficient biomarker function (13).

Nanodiagnostics is an emerging field of research in which NPs are intentionally introduced into the human body. The enormous surface-to-mass ratio of NPs of interest is of vital importance for the study of surface effects of amyloid peptides (14). Surface effects have been proposed for amyloid protein assembly *in vivo*, as they may explain why amyloid proteins misfold at concentrations that are insufficient for peptide fibril formation in solution *in vitro*. The surfaces of the NPs provide an external constraint to the aggregation of these peptides and thus may act to catalyze the aggregation process, where the surface itself may accelerate or inhibit amyloid peptide accumulation depending on the intrinsic propensity for amyloid peptide incorporation into solution (15).

In figure 2, the expression of biomolecule binding to a surface is due to a balance between adsorption energy gain and entropy loss. Physisorption is the main mechanism driving the coating of inorganic surfaces by biomolecules in biological media. It is based on the attractive forces present between the protein or peptide to the surface. The forces leading to physisorption are electrostatic (Coulomb) interactions between opposite charges, hydrogen bond formation and Van-der-Waals interactions. Some amino acid side chains (e.g. thiols) can also be chemisorbed to surfaces leading to essentially covalent binding of the peptide. Tuning the properties of the protein by changing its sequence can lead to very high affinities towards a specific surface. Such highly optimized peptides are used, for example, to coat the surface of implants to improve their biocompatibility. Surface coatings prevent denaturation of native protein structures (16).

Therefore, magnetite is a very dense, fragile, hard mineral with ferromagnetic properties, capable of attracting iron and steel along with other metals. Any magnetic field is a consequence of a flow of electrons, its strong magnetism is due to a phenomenon of ferrimagnetism: the magnetic moments of the different iron cations of the system are strongly coupled, by anti ferromagnetic interactions, but in such a way that in each united cell there is an uncompensated magnetic moment. The sum of these uncompensated magnetic moments, strongly coupled to each other, is responsible for magnet

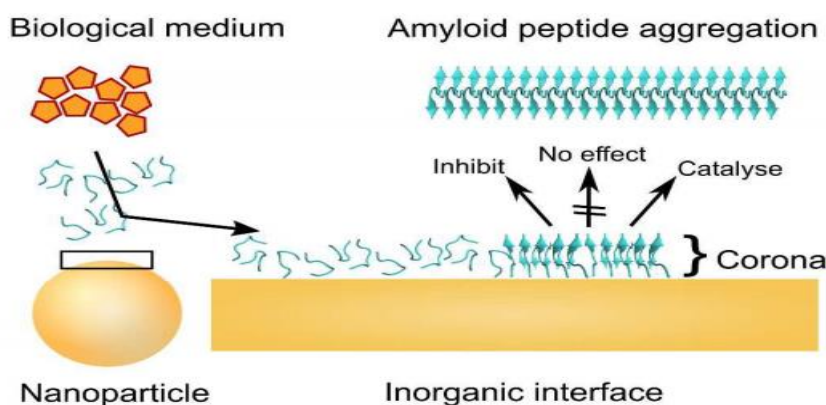


Figure 2. Schematic description: Effects of non-biological media (inorganic solid surfaces or NPs, shown in yellow) on the aggregation rate of amyloid peptides or proteins (shown in cyan). Upon contact with a biological medium, the surfaces of the inorganic NPs become coated with a biofilm, called a "corona". The surface of the NPs and the resulting corona can determine whether the aggregation of the amyloid peptide is influenced, e.g. catalysed or inhibited (16).

ite being a magnet. In Colombia, alluvial type mining, the excavation modality is vertical, approximately 5g of gold is extracted daily, as last is the separation of magnetite from gold, this is totally wasted, without counting that it contains interesting amounts of gold in NPs that can be well exploited (17).

On the other hand, the most common disorders nowadays are: Parkinson's disease, Alzheimer's disease, prion diseases and amyotrophic lateral sclerosis, which can be classified as proteinopathies, synucleinopathies, amyloidopathies and tauopathies, respectively; therefore, NPAu have a potential capability as a possible diagnostic of pathological protein aggregation, this could be a promising approach in the treatment of such diseases. NPs can promote or inhibit protein aggregation, depending on coating, shape, size, surface charge and concentration. A variety of common pathogenic features have been identified, such as genetic and environmental factors, however, the most common feature of all these diseases is protein misfolding in specific regions of the brain; they are identified as the intra- or extracellular accumulation of aggregates in the central nervous system that are abundant in  $\beta$ -sheets (17).

Amyloidosis is known as a clinical disorder resulting from extracellular deposits of amyloid fibrils *in vivo*. Amyloid fibrils are highly symmetrical elongated protein aggregates that share a pattern of characteristics for their quaternary structure. These fibrillar structures, are associated with numerous diseases, including Alzheimer's disease, ( $A\beta$ -peptide, tau protein), spongiform encephalitis (prion proteins), type 2 diabetes (human islet amyloid polypeptide, hIAPP) and Parkinson's ( $\alpha$ -synuclein). Amyloid peptides are soluble in their native state and only aggregate under specific circumstances. Whether aggregated fibrils or soluble intermediates are toxic in the development of the associated diseases is still under investigation.

One of the hypotheses that amyloid beta peptides ( $\beta A$ ) of Alzheimer's disease behave like prions was published in a paper using an experimental model with transgenic mice. In these the expression of the mutant forms was controlled by the promoter of the gene encoding for glial fibrillary acidic protein (GFAP) and were coupled to the gene encoding for the enzyme

luciferase, in order to detect and monitor in time the brain bioluminescence signals. This was confirmed by Western blot assays, enzyme-linked immunosorbent assays and immunocytochemistry, which showed a relative increase in amyloid protein, an increase in GFAP protein and a bilateral distribution of amyloid in both hemispheres of the forebrain (5).

On the other hand, there is also experimental evidence that inclusions or Lewy bodies, whose main component is  $\alpha$ -synuclein, are present in Parkinson's disease, and that the development of these intracellular aggregates by misfolding of  $\alpha$ -synuclein can spread cell to cell. These results and others have led to the hypothesis that a prion-like mechanism exists to explain the spread of  $\alpha$ -synuclein in the nervous system (18).

The theory of exosomes in the propagation of NEs is given by the abnormal aggregation of proteins that has been implicated in neurodegenerative processes. The transmission of these protein aggregates between neurons is the mechanism underlying the progression and pathophysiology of diseases. However, the precise mechanism by which these aggregates are transmitted between neurons is unknown. Preformed protein aggregates are the seeds of more complex protein aggregates, which occur during the lag phase of the cell cycle, a molecular principle that underlies prion infectivity and transmissibility, and thus may apply to the interneuronal transmission of protein aggregates. The mechanism dependent on preformed proteins has not been fully tested; but another possibility is that protein aggregates may be propagated by interneuronal transfer, although this mechanism is not well defined. Exosomes play important roles in the interneuronal transmission of pathogenic proteins in neurodegeneration (10).

The spectrum of clinical signs of these diseases, when fully developed, include pyramidal (spastic paresis with pyramidal tract signs) and extrapyramidal dysfunction, and akinetic mutism and unresponsiveness.

The AuNP system is based on the monitoring of protein structural changes, i.e. the biomarkers exhibit specific receptors that bind to the abnormal protein portions allowing the visualization of protein interactions with the NPs. This technique is characterized by its high sensitivity and specificity in the early determination of ENs

in vitro. To understand the interaction between the AuNP and the abnormal amyloid aggregate-forming proteins, it is necessary to know that the proteins present in the serum sample induce the formation of a corona that covers the surface of the AuNP, this biocompatibility is due to the fact that the instrument contains specific markers for such recognition.

To understand the interaction between the AuNP and the abnormal amyloid aggregate-forming proteins, it is necessary to know that the proteins present in the serum sample induce the formation of a corona that covers the surface of the AuNP, this biocompatibility is due to the fact that the instrument contains specific markers for such recognition.

The center of the sphere is composed of magnetite; ferrous-differric oxide, which has magnetic properties that are used as affinity probe with misfolded proteins, such aggregation occurs on the surface of the nanodevice which is specifically directed to the region of the C-terminal domain of the protein. The experimental model presents important properties on its surface, given the resulting force of attraction between the peptide and the surface of the AuNP, forming an amyloid aggregation effect; this also implies that a secondary ordering mechanism on the surface of the NPs is determined by the rate of formation of fibrils.

On the other hand, these assays were carried out with the exposure of the AuNP to a solution of amyloidogenic peptides with a specific concentration; the objective was to cover the NPs with these proteins. Once the contact of the proteins with the AuNP was formed, the suspension was observed. In the substrate, a higher intensity of detection was evidenced in the C-terminal domains of the anomalous protein, since the receptors of the NPs recognized the protein and acted as a marker for the detection of the structures related to the target point to be analyzed. It should be taken into account that NPs are unstable at certain pH values or ionic strengths of electrolytes depending on their nature (19).

## DISCUSSIONS

NPs can hinder the self-assembly of peptides into amyloid fibrils by binding to specific peptide fragments and thus inhibiting their aggregation. The  $\beta$ -sheet tendency interacts easily with the

gold surface, whereas other conformations, such as  $\alpha$ -helices, needed to be deflected.

Simulations suggest that AuNP binds specifically to the C-terminal fragment region; this implies that for all peptides, initial contact through a charged group with the gold surface leads to an upward concentration and local alignment of peptide monomers on the surface. This alignment is the potential cause of the formation of a first layer of  $\beta$ -sheet-rich oligomers. In all cases, the N-terminus shows greater alternation than the C-terminus, indicating a binding of the C-terminus to a surface, this binding on the surface occurs on a short time scale (fig. 3) (16).

To study this prototype AuNP-based biomarker and its applications to treat ENs as a diagnostic targeting amyloid fibrils, the methodology in which NPs are developed that supports an immediate binding of the amyloid peptide to the gold sensor surface, after peptide adsorption, sensors detect and quantify must be elucidated. The peptide molecules form a rigid layer on the gold surface, suggesting tight binding and structure formation on the surface (fig. 4).

Data from different sources claim that the hydrophobic C-terminal sequence of A $\beta$  folds into a  $\beta$  conformation, and that A $\beta$  fibrils are composed of multiple  $\beta$  units that polymerize in a parallel, in-register orientation (19).

Regarding the relationship between amyloid proteins in different neurodegenerative pathologies, it has been proposed that common neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease and their amyloidogenic proteins can self-replicate as prions, with a neurotoxic profile in the nervous system and are considered as prion-like diseases. In fact, Tau, B-amyloid and  $\alpha$ -synuclein have the ability to spread cell to cell (5).

Although  $\beta$ A aggregates and  $\alpha$ -synuclein aggregates behaved similarly to prions in experimental models, there is currently no evidence that Alzheimer's disease and Parkinson's disease are contagious, in the sense of being transmissible between humans.

In addition to this, prion diseases or spongiform encephalopathies are a family of rare neurodegenerative pathologies, the common characteristic of these diseases is that their etiology is

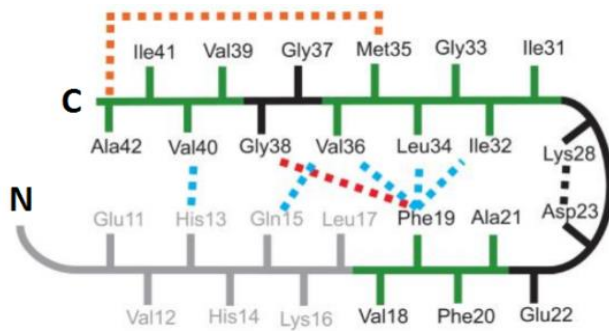


Figure 3. Sequence and structure of the monomeric unit in amyloid fibrils (20).

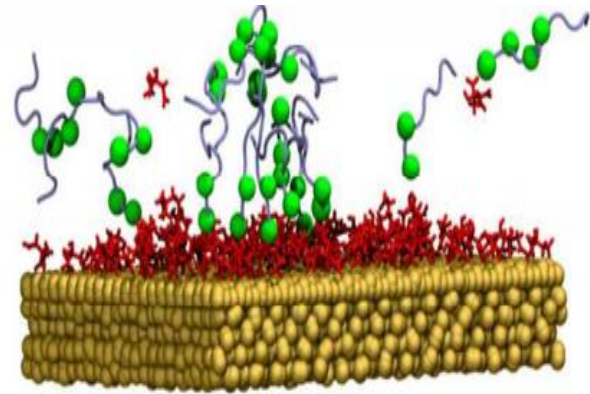


Figure 4. Simulation: Gold surfaces (gold) assembled with specific receptors for A $\beta$  (red) interacting with peptide monomers that bind to the gold surface. C-terminal receptors are depicted as a green sphere to illustrate favored C-terminal binding of the peptide (blue) to the NPAu surface stabilized by iron oxide-rich magnetite cores (16).

linked to misfolding and aggregation of a host protein. The most common form in humans is Creutzfeldt-Jakob disease (CJD), which has been classified as sporadic (eCJD), familial (fCJD), iatrogenic (iCJD) and new variant (nvCJD). eCJD is the most frequent, constituting about 85% of the cases of prion diseases. More precisely, prion diseases are fatal diseases belonging to the

group of ENs of the brain of animals and humans. The normal isoform of the prion protein has been identified in mammalian tissues, including sheep, cattle, hamsters, mice and humans, with 80-90% homology between species and 98% homology in sheep and cattle prion genes, which would explain the interspecies barrier passage event (21).

## CONCLUSIONS

1. The advances of nanotechnology in health are dedicated to the design of new diagnostic methods, such as AuNP for the early estimation of ENs. For such reason, these NPs are functional to demonstrate the existence of an anomalous structure recognized and exhibited by the same, which is highly sensitive and specific, since the fused receptor acted as a sensor for the detection of prion structures. Therefore, further research studies are required to determine the molecular mechanisms in which the active functioning of the nanostructures is developed, in order to provide different target points in the treatment of various biological particles that would be involved in the progression of such diseases.
2. Since this technique is being used as a possible evaluation in the progression of NDs, it is useful for the possible generation of treatments, which are focused on the reversion of the nervous system symptomatology and the decrease of isolated amyloid plaques in the brain. Some authors propose that the implementation of these molecular biomarkers does not generate side effects in the patient, since they are not neurotoxic and are biocompatible with the normal metabolism in the organism.
3. At present, there is much knowledge about the clinical characteristics and pathogenesis of the diseases mentioned above, correlated with their alterations at the molecular level. However, with the advent of biotechnology today, it has been possible to design techniques and technologies with NPs that allow the identification of specific mutations and provide a diagnosis in individuals at risk of being a carrier of a neurological disorder. Taking into account the research models concerning AuNP, it is possible to infer that the capabilities that make them representative focus on their magnetism and biofunctionality with different biological molecules, in this case, by binding

specifically to amyloid peptides and other ligands directed to a C-terminal cysteine domain present in the protein, which is the major component of amyloid plaques used in these studies.

### CONFLICT OF INTERESTS

All authors declare that they have no competing interests.

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## PRIMUL STUDIU NAȚIONAL DE PREVALENȚĂ DE MOMENT A INFECȚIILOR ASOCIATE ASISTENȚEI MEDICALE ȘI A CONSUMULUI ANTIMICROBIENELOR ÎN SPITALELE DIN REPUBLICA MOLDOVA

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**Keywords:** *point prevalence survey, healthcare-associated infections, antimicrobial use.*

**FIRST NATIONAL POINT PREVALENCE SURVEY OF HEALTHCARE-ASSOCIATED INFECTIONS AND ANTIMICROBIAL USE IN ACUTE CARE HOSPITALS IN THE REPUBLIC OF MOLDOVA**

**Introduction.** HAIs present a major public health problem with an impact on morbidity, mortality and quality of life. The objective of the study was to highlight the problem of HAIs and AM use, identification of risk factors and raising awareness of the phenomenon.

**Material and methods.** The methodology of the PPS was patient-based, developed based on ECDC Protocol 5.3/2016. The study was accomplished in 2018. The sample of the PPS included 67 hospitals, 546 wards and 10594 patients.

**Results.** The prevalence of HAIs was 1,6%, with predominance of pneumonia (25%), SSI (16.1%), LRI (14.9%), UTI (11.3%) and varies depending on the wards profile, clinical diagnosis, length of hospital stay and risk factors (medical device, surgery). HAIs were caused by *Klebsiella* spp. in 26%, *Enterococcus* spp. – 18% and coagulase-negative *Staphylococci* – 14%, *P. aeruginosa* – 12%. *Klebsiella* spp. presented resistance to 3rd generation cephalosporins in 84.6% and to carbapenems – 46.2%. The prevalence of AM use was 42.7%, for the group of 3rd generation cephalosporins – 34.5%. More frequently antibiotics were administered for treatment (73.8%), and for surgical prophylaxis >1 day in 93.5%.

**Conclusions.** Data on HAIs and AM use in hospitals, first obtained by implementing active surveillance based on the ECDC tool, are standardized and comparable at national and international level and allow assessing the situation in hospitals, obtaining evidence for infection prevention and control and AM stewardship programmes at local level, as well as reasoning the national policies.

**Cuvinte cheie:** *studiul de prevalență de moment, infecții asociate asistenței medicale, consumul de antimicrobiene.*

**Introducere.** IAAM prezintă o problemă majoră de sănătate publică cu impact asupra morbidității, mortalității și calității vieții. Obiectivul studiului a constat în elucidarea problemei IAAM și a consumului AM, factorilor de risc și sporirea conștientizării fenomenului.

**Material și metode.** Metodologia PPS a fost bazată pe pacient, elaborată în baza Protocolului ECDC 5.3/2016. Studiul s-a realizat în a. 2018 pe un eșantion de 67 de spitale, 546 de secții și 10 594 de pacienți.

**Rezultate.** Prevalența IAAM a constituit 1,6%, predominând pneumoniile (25%), SSI (16,1%), LRI (14,9%) și UTI (11,3%) și a variat în funcție de profilul secției, diagnosticul de bază, durata spitalizării și factorii de risc (inserat dispozitiv medical, intervenție chirurgicală). IAAM au fost cauzate de *Klebsiella* spp. în 26% cazuri, *Enterococcus* spp. – 18%, *Staphylococci* coag. negativ – 14%, *P. aeruginosa* – 12%. *Klebsiella* spp. a prezentat rezistență la cefalosporine de generația a 3-a în 84,6%, la carbapeneme – 46,2%. Prevalența consumului AM a constituit 42,7%, cefalosporinele de generația a 3-a – 34,5%. Cu scop de tratament AM au fost administrate în 73,8%, în profilaxia chirurgicală >1 zi – 93,5%.

**Concluzii.** Datele privind IAAM și consumul AM în spitale, obținute în premieră prin implementarea supravegherii active în baza instrumentului ECDC, sunt standardizate și comparabile la nivel național și internațional și permit evaluarea situației în spitale, obținerea dovezilor pentru programele de prevenire și control a IAAM și utilizarea rațională a AM la nivel local și argumentarea politicilor naționale.

**ABREVIERI:** *HAI*s/*IAAM*, healthcare-associated infections/infecții asociate asistenței medicale; *AM*, antimicrobiene; *PPS*, studiul de prevalență de moment a infecțiilor asociate asistenței medicale și consumul antimicrobielenelor în spitale; *ECDC*, Centrul European de Prevenire și Control al Bolilor; *SSI*, infecție de situs chirurgical; *LRI*, infecție a căilor respiratorii inferioare; *UTI*, infecție a tractului urinar; *UE*, Uniunea Europeană; *RAM*, rezistență antimicrobiană; *PCI*, programe de prevenire și control al infecțiilor; *ATI*, terapie intensivă; *AHR*, dozatoare pentru antiseptic; *PVC*, cateter vascular periferic; *CVC*, cateter vascular central; *SEE*, Spațiul Economic European.

## INTRODUCERE

Infecțiile asociate asistenței medicale (IAAM) constituie unul din cele mai frecvente evenimente adverse în acordarea asistenței medicale și o problemă majoră de sănătate publică cu impact asupra morbidității, mortalității și calității vieții (1, 2). În medie, în orice moment, până la 7% dintre pacienți din țările cu venituri mari și 10% din țările cu venituri mijlocii și mici, contactează cel puțin o IAAM. Decesele provocate de IAAM constituie circa 10% dintre pacienții afectați (1).

Estimările privind IAAM în Uniunea Europeană (UE) au elucidat că peste 4 milioane de pacienți sunt afectați anual de aproximativ 4,5 milioane de episoade de IAAM, ceea ce duce la 16 milioane zile suplimentare de spitalizare, 37 mii decese și contribuie la încă 110 mii decese. În UE peste 380 mii persoane anual fac infecții provocate de bacterii rezistente la antibiotice, iar 25 mii de persoane decedază anual din cauza eșecului terapeutic (2).

În Republica Moldova în sistemul național de supraveghere epidemiologică al bolilor transmisibile, IAAM și rezistența antimicrobiană (RAM) sunt listate ca probleme speciale de sănătate publică (3). Cadrul normativ național prevede exigențe privind supravegherea, prevenirea și controlul eficient al IAAM. Concomitent, IAAM sunt subraportate în Republica Moldova și nu permit aprecierea situației reale și identificarea factorilor de risc. Doar prin cunoașterea intensității fenomenului IAAM, în conjuncție cu monitorizarea consumului antimicrobielenelor (AM) și supravegherii RAM, pot fi identificate soluții pentru prevenirea și combaterea acestora și sporirea calității actului medical și siguranței pacienților (4, 5, 6).

Literatura de specialitate relevă, că IAAM pot fi prevenite cu 30% prin implementarea programelor eficiente de prevenire și control al infecțiilor (PCI), iar supravegherea IAAM contribuie la diminuarea cu 25-57% (1, 4, 5, 6). Una din componentele de bază a programelor PCI constituie supravegherea IAAM, fiind crucială la nivelul

instituției medico-sanitare pentru a ghida intervențiile PCI și pentru a detecta izbucnirile (4, 5, 6). Regulamentul Sanitar Internațional (2005) poziționează PCI eficiente drept o strategie cheie în gestionarea amenințărilor cu care se confruntă sănătatea publică la nivel internațional (7, 8).

*Obiectivul studiului* a constat în elucidarea problemei infecțiilor asociate asistenței medicale și a consumului antimicrobielenelor în spitale, identificarea factorilor de risc și sporirea conștientizării problemei la lucrătorii medicali și factorii de decizie prin utilizarea metodelor active de supraveghere epidemiologică.

## MATERIAL ȘI METODE

Metodologia studiului de prevalență de moment a infecțiilor asociate asistenței medicale și consumului antimicrobielenelor în spitale (PPS) a fost elaborată în baza Protocolului 5.3/2016 dezvoltat de Centrul European de Prevenire și Control al Bolilor (ECDC) (9), bazată pe pacient, incluzând prevenirea și controlul IAAM și principalele variabile din ESAC (European Surveillance of Antimicrobial Consumption Network). PPS a fost efectuat în 67 de spitale publice și private, 546 de secții și a inclus un eșantion de 10 594 de pacienți eligibili. Echipele instituționale au colectat datele pe formulare standard în perioada noiembrie-decembrie 2018. Validarea datelor a fost efectuată aleatoriu de echipe externe de epidemiologi din Agenția Națională pentru Sănătate Publică. Analiza datelor a fost realizată cu utilizarea programului Helics.Win.Net.

Studiul PPS a generat indicatori de spital, secție și pacient, inclusiv prevalența IAAM și a consumului de AM în raport cu factorii de risc. *Indicatorii de spital și secții* includ variabile: caracteristicile spitalului/secției (tipul, dimensiunea, profilul), durata medie de spitalizare, măsuri administrative de control al infecțiilor (planificare, analiză, strategii multimodale), infrastructura (condiții de izolare), asigurarea cu resurse umane, asigurarea cu echipamente și consumabile pentru controlul infecțiilor, monitoringul microbiologic și compliance la protocoale.





*Indicatorii pentru pacient* includ variabile privind caracteristicile pacientului, factorii de risc pentru IAAM, prevalența IAAM și consumul AM, rezistența la antimicrobiene a agenților cauzali a IAAM.

## REZULTATE

### *Indicatorii de spital și secții*

În structura instituțiilor medico-sanitare spitalicești incluse în studiu (67 de spitale), prevalează spitalele de tip primar cu 50,7%, urmate de cele de tip terțiar – 23,9% și secundar – 19,4%. Mărimea medie a spitalelor constituie 254,8 paturi. Secțiile de profil terapeutic prevalează cu 31,9%, urmate de secțiile chirurgicale cu 24,4% și cele de terapie intensivă (ATI)/Reanimare/STROKE – 11,5%.

În studiu au fost incluse 94,7% paturi, dintre care 3,9% – paturi ATI/Reanimare/STROKE.

Durata medie de spitalizare a pacienților o constituie 9,5 zile, variind de la 1,1 zile până la 64,2 zile per spital. În 70,1% din spitale durata medie de spitalizare constituie 4-7 zile.

Planificarea măsurilor de prevenire și control a infecțiilor se realizează de către spitale anual, care în 100% dispun de planuri instituționale de supraveghere și control al IAAM, iar rapoarte anuale privind analiza IAAM au fost elaborate în 77,6% spitale.

Prezența celor 7 componente a strategiei multimodale de prevenire și control al IAAM prioritare și consumul rațional a AM nu au fost raportate de către spitale. Cele mai consistente date au fost atestate pentru controlul pneumoniilor la următoarele componente: „Protocoale instituționale”, „Instruire” și „Supraveghere”. Cele mai limitate date per componentele strategiei multimodale sunt atestate pentru următoarele IAAM prioritare: septicemie, infecțiile site-urilor chirurgicale; consumul rațional de antimicrobiene. Doar un spital (1,5%) a raportat prezența procedurii oficializate pentru consumul AM (antimicrobial stewardship).

Analiza asigurării cu resurse umane pentru organizarea planificării, implementării, monitorizării și evaluării măsurilor de prevenire și control a IAAM și RAM relevă, că în 71,6% dintre spitale lipsește medicul epidemiolog. Asigurarea cu medic epidemiolog de spital constituie 0,3 pentru 250 de paturi. Asistenți medicali dedicați

controlului infecțiilor sunt angajați în circa jumătate dintre spitale (53,7%). Media specialiștilor dedicați controlului infecțiilor constituie 0,8 pentru 250 de paturi, variind de la zero până la 7,4%. Prezența specialistului dedicat politicii consumului rațional al antimicrobienelelor a fost raportat de 3 spitale (4,5%).

Numărul mediu de asistenți medicali în secțiile ATI/Reanimare/STROKE pentru un pat constituie 1,7 și variază de la 0,4 până la 4,0. Numărul mediu al infirmierilor în secțiile ATI/Reanimare/STROKE pentru un pat constituie 0,9 cu variabile minime de 0,25 și maxime – de 1,8.

Pentru asigurarea izolării pacienților, 95,5% dintre spitale dispun de saloane cu un pat, iar 68,7% – de saloane cu un pat dotate cu WC și duș individual. Rata medie a saloanelor cu un pat constituie 12,3% și variază de la zero până la 100%. Rata medie a saloanelor cu un pat dotate cu WC și duș individual este de 5,2 %, variind între 0-100%. Dispun de saloane pentru izolarea pacienților cu infecții respiratorii – 2 spitale (3,0%).

Prezența paturilor dotate cu dozatoare pentru antiseptic (AHR) au raportat 40,3% dintre spitale, rata medie a paturilor dotate cu astfel de dispozitive fiind de 5,1%. În funcție de specialitatea secției rata paturilor dotate cu dozatoare AHR variază de la zero în secțiile cu profil psihiatrie și reabilitare până la 47,6% în secțiile ATI/Reanimare/STROKE (fig.1).

Rata lucrătorilor medicali care dispun de dozatoare portabile cu antiseptic pentru igiena mâinilor variază de la spital la spital. În 53,7% dintre spitale ponderea lucrătorilor medicali asigurați cu astfel de dozatoare constituie 0-25%, și doar în 7,5% dintre spitale – >75% dintre personal este asigurat cu AHR. Media acestui indicator per secții constituie 9,1%, variind de la zero în secțiile cu profil recuperare până la 17,4% în secțiile cu profil neonatologic (fig.1).

Observații directe a procedurii de igienizare/dezinfectie a mâinilor nu au fost efectuate în 64,2% dintre spitale. În medie acest indicator a constituit 12,9 observații per secții/an, variind de la 0 în secțiile de profil geriatric și recuperare până la 17,1 în secțiile de profil chirurgical. Consumul mediu de antiseptic pentru igiena mâinilor per spital constituie 4,1 L/1000 pacient-zile, cu o variație de la 0,0 la 58,8 L/1000 pacient-zile. Mai

mult de jumătate dintre spitalele primare (58,8%) și secundare (76,9%) au raportat un consum de antiseptic sub 3 L/1000 pacient-zile. Secțiile ATI/Reanimare/ STROKE consumă cea

mai mare cantitate de antiseptic per an cu 41,8 L/1000 pacient-zile, urmate de secțiile neonatologice (6,7 L/1000 pacient-zile) și chirurgicale (5,8 L/1000 pacient-zile).

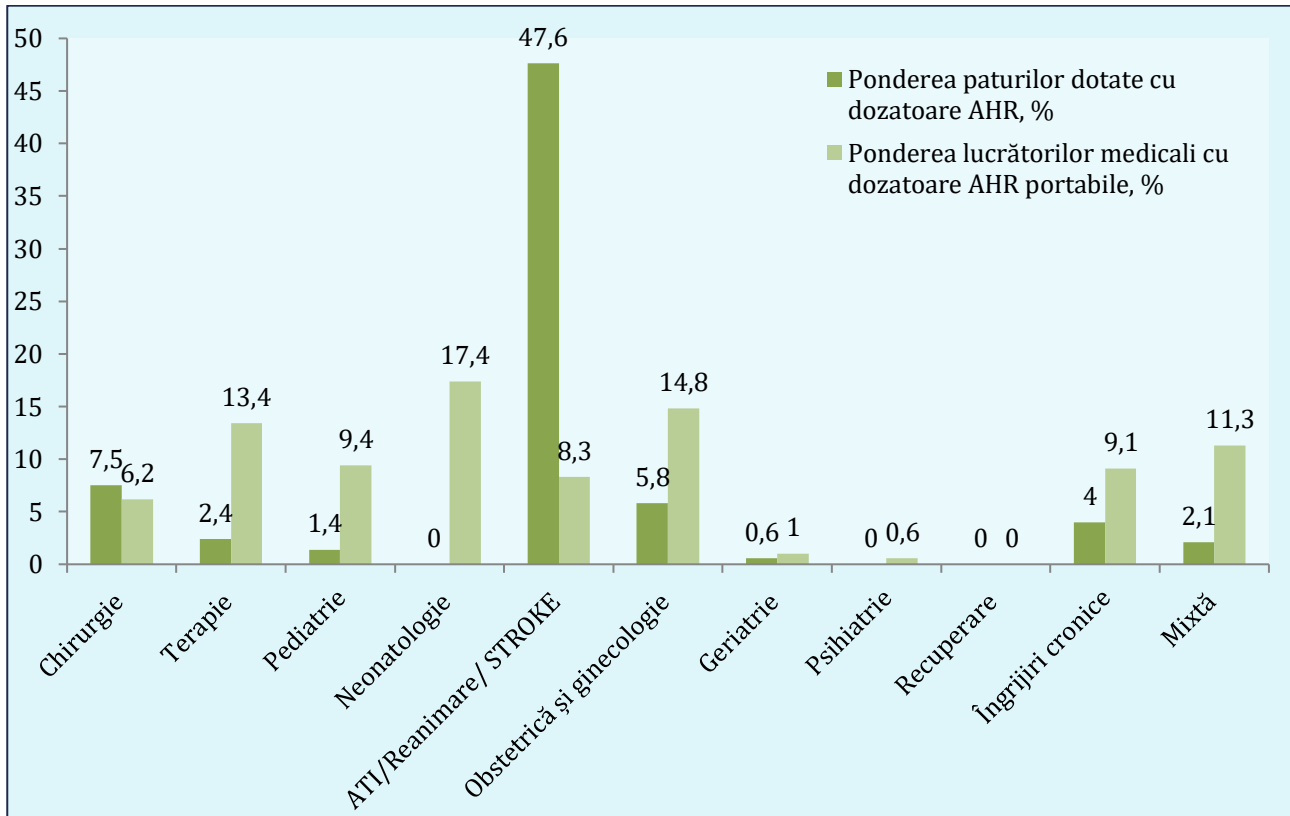


Figura 1. Rata paturilor și a lucrătorilor medicali dotați cu dozatoare AHR în funcție de specialitatea secțiilor, %.

Analiza accesibilității la servicii de laborator microbiologice în zilele de week-end relevă că în ziua de sâmbătă clinicienii pot să solicite teste microbiologice și/sau de screening și să primească rezultatele în 38,8% și respectiv 29,8% spitale, iar în ziua de duminică în 4,5% și respectiv 3,0% spitale. Testarea hemoculturilor nu s-a realizat pe parcursul anului de studiu în 29,8% dintre spitale. Teste de scaun pentru determinarea *Clostridium difficile* au fost efectuate în 3 spitale (4,5%).

**Indicatorii pentru pacient**

Din 10 594 de pacienți eligibili cea mai numeroasă grupă de vârstă este de 18-64 ani cu 54,9%. Vârsta medie a pacienților constituie 46,6 ani. În funcție de masa corporală la naștere al copiilor nou-născuți, rata cea mai mare o alcătuiesc nou-născuții cu masa corporală peste 2500 gr cu 80,1%.

Din numărul total de pacienți, 8,4% au suportat

intervenții chirurgicale invazive, iar intervenții minim invazive – 5,5% pacienți. Rata pacienților, care aveau inserate dispozitive medicale (factori de risc ai IAAM) a constituit: 13,1% – cateter vascular periferic (PVC), 3,3% – cateter urinar, 1,7% – cateter vascular central (CVC) și 0,8% dintre pacienți erau intubați.

**Infecțiile asociate asistenței medicale**

Prevalența IAAM a constituit 1,6% cu variabile de la zero până la 6,1% per spital. Cea mai mare prevalență se atestă în secțiile de ATI/Reanimare/STROKE cu 20,0%, urmată de neonatologie – 2,5%, psihiatrie – 2,1%, chirurgie – 2,0% și obstetrică și ginecologie – 1,9%; media per secții cu excepția ATI/Reanimare/STROKE constituie 1,2%. În structura IAAM topul aparține pneumoniilor cu rata de 25,0%, infecțiilor de situs chirurgical (SSI) cu 16,1%, altor infecții ale căilor respiratorii inferioare (LRI) cu 14,9% și infecțiilor sistemului urinar (UTI) cu 11,3% (fig.2).

În structura pneumoniilor predomină pneumoniile fără confirmare microbiologică care constituie 69,0%, similar în infecțiile urinare

predomină cele fără confirmare microbiologică cu 52,6%. În structura SSI predomină infecțiile de organ/cavitate cu 44,4%.

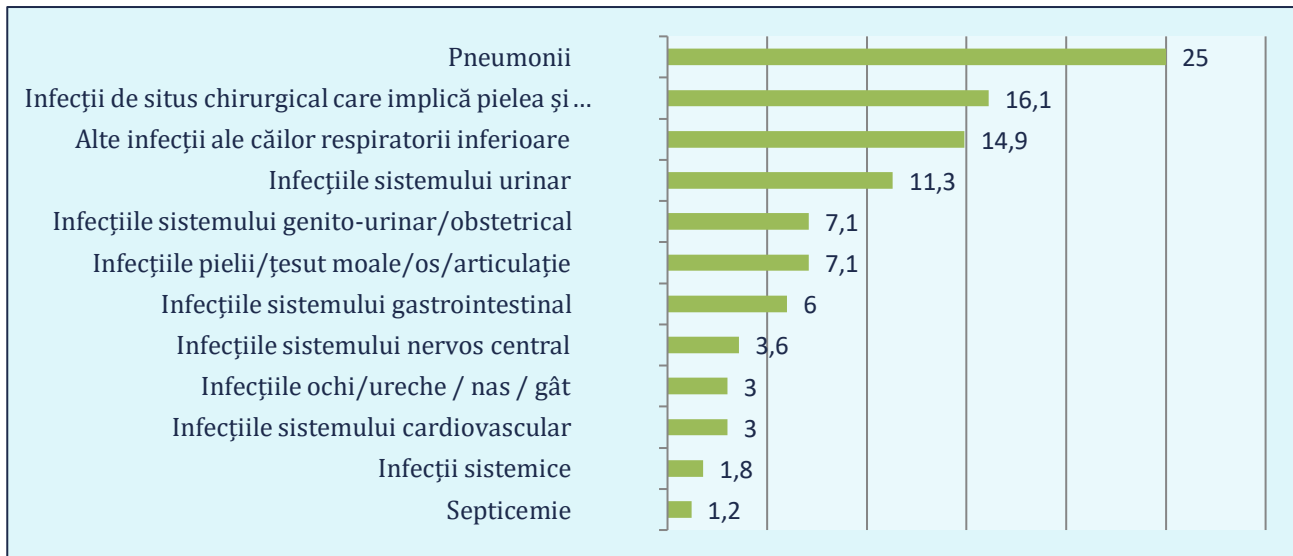


Figura 2. Structura IAAM, %.

Diagnosticul etiologic al IAAM a fost stabilit în 23,2% din cazuri, predominând microorganismele gram negative cu o pondere de 58%, urmate de microorganismele gram pozitive cu 38% și fungi cu 2,0%. În structura microorganismelor izolate de la pacienți cu IAAM predomină *Klebsiella spp.* cu ponderea de 26%, *Enterococcus spp.* – cu 18% și *Staphylococci coag. negativ* cu 14%, urmate de *Pseudomonas aeruginosa* cu 12%. *Klebsiella spp.* mai frecvent a fost raportată ca agent etiologic în pneumonii (37,5%), SSI (37,5%) și UTI (40,0%). Se atestă o rezistență

sporită a *Klebsiella spp.* la cefalosporine de generația 3-a (84,6%) și la carbapeneme (46,2%) și a *Pseudomonas aeruginosa* la carbapeneme (66,7%).

Prevalența IAAM la pacienții cu boli rapid fatale și în faza terminală depășește media de 4 ori și constituie 8% și 7% respectiv. Pacienții cu intervenții chirurgicale invazive fac IAAM de circa 3 ori mai frecvent, prevalența IAAM constituind 6,9%. La pacienții cu dispozitive medicale inserate se observă o prevalență a IAAM mai mare decât media (1,6%). Astfel, prevalența IAAM la pacienții intu-

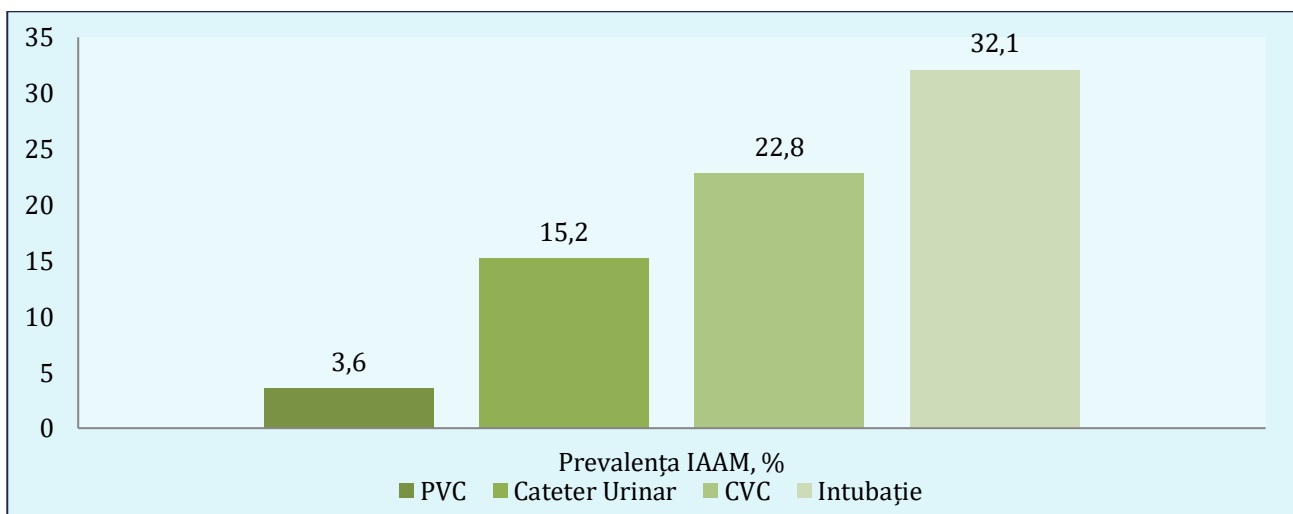


Figura 3. Prevalența IAAM în funcție de tipul dispozitivului medical inserat, %.

bați constituie 32,1%, la pacienții cu cateter vascular central – 22,8%, la pacienții cu cateter urinar – 15,2%, iar la cei cu cateter vascular periferic – 3,6% (fig.3).

Structura IAAM în funcție de origine (locul și timpul infectării) este prezentată prin infecții asociate cu spitalizarea actuală în 72,0%, prezente la internare – 28,0%. Din numărul de IAAM prezente la internare 51,1% sunt asociate cu alt spital și 34% sunt asociate cu o spitalizare anterioară în același spital, în 14,9% nu s-a identificat locul infectării. IAAM cel mai frecvent au apărut la 8-14 zile de la spitalizare cu o pondere de 33,1%. Prevalența IAAM crește odată cu creșterea duratei de spitalizare de la 0,6% la pacienții cu durata de spitalizare 1-3 zile până la 3,3% la pacienții cu durata de spitalizare  $\geq 15$  zile.

### **Consumul antimicrobielor**

Prevalența consumului de antimicrobiene constituie 42,7%, în medie la un pacient sunt administrate 1,3 antimicrobiene. Cel mai frecvent AM sunt administrate pacienților cu scop de tratament – 73,8%. Infecțiile comunitare în structura indicațiilor pentru administrarea AM cu scop terapeutic prevalează și constituie 94,1%, urmate de IAAM cu o pondere de 5,9%. Mai frecvent AM cu scop terapeutic au fost prescrise pentru tratamentul infecțiilor sistemului respirator cu o pondere de 47,3%, infecțiile sistemului urinar – 12% și infecțiile ochi/ureche/nas/gât cu 8,8%. În tratamentul IAAM, la fel, mai frecvent AM au fost prescrise în infecțiile sistemului respirator cu o pondere de 41,4%, infecțiile de situs chirurgical care implică pielea și țesut moale dar nu cel osos – 18,2% și infecțiile sistemului urinar și a sistemului genito-urinar/obstetrical – cu 10,1% fiecare. În structura administrării AM cu scop de profilaxie chirurgicală prevalează administrarea AM mai mult de o zi cu o pondere de 93,5%.

Schemele de tratament AM au fost modificate în 5,8%, inclusiv prin escaladare – 5,7%. Calea parenterală de administrare a AM prevalează și constituie 84,9%. În funcție de durata de administrare a AM 94,0% din preparate au fost administrate pe durata de 1-7 zile.

În structura AM consumate predomină cefalosporinele de generația a 3-a cu o pondere de 34,5%, urmate de peniciline cu spectrul larg de acțiune – 12,9% și cefalosporinele de generația 1-a și generația a 2-a cu o pondere de 10,6% și 8,2%, respectiv. Aceste grupe de antimicrobiene prevalează și

în cazul administrării cu scop de tratament, respectiv – 34,8%, 16,1%, 9,7% și 8,4%. În profilaxia chirurgicală rata cefalosporinelor de generația a 3-a prevalează constituind 43,3%, cefalosporinele de generația 1-a se plasează pe locul doi cu 18,8%, fiind urmate de derivații de imidazol cu 11,7% și cefalosporinele de generația a 2-a cu 10,4%.

Pacienții cu intervenții chirurgicale invazive mai frecvent consumă AM cu o prevalență de 99,0%. Prevalența consumului AM la pacienții cu dispozitive medicale depășește media de 42,7%, constituind 126,1% la pacienți cu CVC, 96,4% la pacienți cu PVC, 120,4% la cei cu cateter urinar și 117,3% la persoanele intubate.

### **DISCUȚII**

Studiul național de prevalență de moment a IAAM și consumul AM (noiembrie-decembrie a. 2018), printr-o investigație transversală bazată pe pacient în toate spitalele din țară, a furnizat în premieră date standardizate și a permis calcularea indicatorilor de prevalență a IAAM și a consumului de AM, descrierea factorilor de risc asociați, fiind comparabile la nivel local (instituțional) și în contextul regional/global.

Prevalența IAAM în primul studiu național a constituit 1,6%, comparativ cu 13,3% determinată în studiul PPS pilot realizat în 5 spitale naționale în martie 2018 (10, 11). La nivel regional în primul studiu PPS cu participarea țărilor UE/SEE, realizat de ECDC în perioada 2011-2012, s-a stabilit prevalența IAAM de 6,0%, cu o variație de 2,3%–10,8% între țări (12). Diferența datelor obținute poate fi determinată de experiența țărilor în utilizarea metodelor active de supraveghere, implementarea definițiilor de caz a IAAM și IAAM active, tipul spitalelor și profilul secțiilor incluse în studiu, facilități și echipamente pentru complianța la protocoale, complexitatea programelor PCI și politicilor naționale.

La nivel național cea mai mare prevalență a IAAM se atestă în secțiile ATI/Reanimare/ STROKE cu 20% și media pentru celelalte secții este de 1,2%, comparativ cu 19,5% și respectiv 5,2% în studiul PPS ECDC. Astfel, evidențiem că complexitatea și severitatea bolii de bază și prezența factorilor de risc multipli în secțiile ATI/Reanimare/STROKE, intervențiile chirurgicale invazive, inserarea dispozitivelor medicale, durata prelungită de spitalizare contribuie la dezvoltarea IAAM, determi-

nând o prevalență sporită comparativ cu media.

Datele privind structura IAAM la nivel național sunt comparabile cu datele ECDC, ponderea majoră fiind reprezentată de pneumonii – respectiv 25% și 19,4%; SSI – 16,1% și respectiv 19,6%; UTI – 11,3% și 19%.

Confirmarea etiologică a IAAM a fost raportată doar în 23,2% dintre cazuri comparativ cu media europeană de 54,1%, fiind influențată de implementarea parțială a protocoalelor clinice naționale, de asemenea de accesibilitatea limitată la serviciile de laborator microbiologice în zilele de week-end. Datele studiului național au evidențiat cele mai frecvente microorganisme în etiologia IAAM: *Klebsiella spp.* – 26%, *Enterococcus spp.* – 18% și *Staphylococci coag. negativ* – 14%, urmate de *Pseudomonas aeruginosa* – 12%. *Klebsiella spp.* este cel mai frecvent agent cauzal în pneumonii (37,5%), SSI (37,5%), UTI (40%), cu o rezistență sporită la cefalosporine de generația a 3-a (84,6%) și carbapeneme (46,2%). *Pseudomonas aeruginosa* prezintă rezistență sporită la carbapeneme (66,7%). Studiul ECDC evidențiază rate sporite a rezistenței la cefalosporine de generația a 3-a (53%) pentru *Klebsiella spp.*, la carbapeneme cu 19,3% și 31,8% respectiv pentru *Klebsiella spp.* și *P. aeruginosa*.

Prevalența consumului de AM în studiul PPS național constituie 42,7%, comparativ cu 35% în țările UE/SEE. Mai frecvent AM sunt administrate cu scop de tratament (73,8% versus 68,4% în țările UE/SEE), iar în scop de profilaxie chirurgicală mai mult de 1 zi constituie 93,5% sau de 1,6 ori mai mult decât în studiul ECDC (59,2%). În structura AM consumate prevalează cefalospo-

rinele de generația a 3-a cu 34,5% versus 9,6% în țările UE/SEE (PPS ECDC). Calea parenterală de administrare a antimicrobienuelor predomină cu 84,9% la nivel național și 70,6% în studiul ECDC (10, 12).

Indicatorii de structură și de proces pentru PCI calculați la nivel de spital au inclus: consumul de antiseptic (L/1000 pacient-zile) ca indicator pentru igiena mâinilor, ponderea saloanelor cu un pat pentru izolarea pacienților cu infecții care necesită măsuri sporite PCI și personal dedicat în prevenirea și controlul infecțiilor.

În pofida faptului că toate spitalele au raportat prezența planurilor de prevenire și control a infecției, componentele strategiei multimodale PCI rămân neimplementate la nivel instituțional, inclusiv pe fundalul numărului insuficient de specialiști competenți în domeniul PCI (lipsa acestora în 71,6% spitale) și în consumul rațional al antimicrobienuelor (prezenți în 4,5% spitale).

Condițiile de izolare a pacienților infecțioși, inclusiv cu infecții aerogene, sunt limitate, rata medie a saloanelor cu un pat constituie 12,3% (0-100% per spital), a saloanelor cu un pat dotate cu WC și duș – 5,2% (0-100% per spital). Doar 2 spitale dispun de saloane cu presiune negativă.

Sunt rezerve la asigurarea complianței la protocolul igiena mâinilor. Consumul mediu de antiseptic pentru igiena mâinilor per spital constituie 4,1 L/1000 pacient-zile cu o variație de 0-58,8 L/1000 pacient-zile, fiind atestat un nivel foarte scăzut comparativ cu țările UE/SEE – respectiv media 18,7 L/1000 pacient-zile cu variații de 6-70,1 L/1000 pacient-zile.

## CONCLUZII

1. Realizarea studiului național de prevalență de moment a IAAM și a consumului de AM în spitale a generat indicatori standardizați de structură și de proces în PCI per spital, secție, pacient cu evaluarea situației reale și evidențierea domeniilor pentru prioritizare și fortificare a capacităților naționale și instituționale.
2. Implementarea metodelor de supraveghere activă prin aplicarea periodică a instrumentului PPS va permite determinarea tendințelor privind IAAM și RAM, stabilirea țintelor, identificarea resurselor, implementarea măsurilor cost-eficiente pentru sporirea calității actului medical și siguranței pacienților și evaluarea impactului la nivel instituțional. Datele generate în cadrul studiilor PPS vor furniza dovezi pentru fundamentarea politicilor PCI și RAM și luarea deciziilor argumentate la toate nivelurile.

## CONFLICT DE INTERESE

Autorii n-au declarat conflict de interese.

## MULȚUMIRI ȘI FINANȚARE

Studiul național de prevalență de moment a infecțiilor asociate asistenței medicale și a consumului antimicrobiene în spitalele din Republica Moldova, a.2018 a fost efectuat cu suportul Institutului de Sănătate Publică din Oslo, Regatul Norvegiei în cadrul Global Health Preparedness Program. Autorii își exprimă gratitudinea dnei Hanne Merete Eriksen, dlui Pawel Stefanoff și altor colegi din Institutul de Sănătate Publică din Oslo care au contribuit la realizarea la nivel național a primului studiu PPS.

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## RETROSPECTIVE STUDY ON THE PREVALENCE OF COVID-19 CONFIRMED CASES AND EVIDENCE OF GENDER BIAS IN LIBYA

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**Keywords:** SARS-CoV-2, comorbidity, clinical symptoms, Libya.

**Introduction.** To stop SARS-CoV-2 from spreading, it's crucial to have a solid understanding of the baseline characteristics of demographic variables, clinical symptoms, and comorbidity so that quarantining and testing protocols can be developed. **Material and methods.** A retrospective study was conducted on COVID-19 Laboratory confirmed cases. Data were collected by using kobo toolbox, demographic variables, clinical symptoms, and Comorbidity was considered. Yates-corrected chi2 tests and Multinomial Logistic Regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) of factors and their impact on COVID-19 prevalence. **Results.** A total of 6302 of which 3536(56.1%) men and 2766 (44%) women with a mean age ( $\pm$ SD) of  $44\pm 17.6$  years were included within the study. Clinical symptoms and Comorbidity were significant for both sexes  $p<0.0001$ . Multinomial Logistic Regression confirmed that age  $<55$ , 3 or more symptoms (OR=1.130 CI95% 1.013-1.261) and 3 or more comorbidities (OR=1.035 CI95% 0.942-1.137) were a significant risk factor for COVID-19 prevalence in male patients, among women, age  $85\geq$ , 3 or more symptoms ( $p<0.0001$ , OR=1.995 CI95% 1.335-2.992) and 3 or more comorbidities ( $p<0.0001$ , OR=1.538 CI95% 1.045-2.640) were significant risk factor for COVID-19 prevalence in females. **Conclusions.** Our study suggests that the prevalence of COVID-19 patients and symptoms was higher in men than women. The high prevalence of smoking could have contributed to the high prevalence of COVID-19 among men. Study also suggests that the presence of at least one or combined comorbidities are risk factors of COVID-19 prevalence and a potential risk factor COVID-19 - related outcomes. More efforts should be exercised to protect patients with one or more comorbidities from being exposed to infection.

**Cuvinte cheie:** SARS-CoV-2, comorbiditate, simptome clinice, Libia.

### STUDIUL RETROSPECTIV VIZĂND INCIDENȚA CAZURILOR DE COVID-19 CONFIRMATE ȘI EVIDENȚA BIASULUI DE GEN ÎN LIBIA

**Introducere.** Pentru a stopa răspândirea SARS-CoV-2, este esențial să fie bine înțelese caracteristicile de bază ale variabilelor demografice, simptomele clinice și comorbiditatea, astfel încât să poată fi dezvoltate protocoale de carantină și de testare. **Material și metode.** A fost efectuat un studiu retrospectiv pe cazurile confirmate de laboratorul COVID-19. S-au colectat date prin aplicarea Kobo toolbox, luându-se în considerare variabilele demografice, simptomele clinice și comorbiditatea. Pentru a estima riscul relativ (OR), intervalul de încredere de 95% (CI) al factorilor și impactul acestora asupra prevalenței COVID-19, au fost utilizate Yates corecția pentru testele chi pătrat și regresia logistică multinomială. **Rezultate.** În studiu au fost incluse 6302 persoane, dintre care 3536 (56,1%) bărbați și 2766 (44%) femei, cu vârsta medie ( $\pm$ SD) de  $44\pm 17,6$  ani. Simptomele clinice și comorbiditatea au fost semnificative pentru ambele sexe  $p<0,0001$ . Regresia logistică multinomială a confirmat că vârsta  $<55$ , 3 sau mai multe simptome (OR=1,130 CI95% 1,013-1,261), 3 sau mai multe comorbidități (OR=1,035 CL95% 0,942-1,137) au constituit un factor de risc semnificativ pentru prevalența COVID-19 la bărbați, iar în rândul femeilor, vârsta de 85 de ani  $\geq$ , 3 sau mai multe simptome ( $p<0,0001$ , OR=1,995 CI95% 1,35-2,992), 3 sau mai multe comorbidități ( $p<0,0001$  OR=1,538 CL95% 1,045-2,640) au prezentat factori de risc semnificativ pentru incidența COVID-19. **Concluzii.** Studiul constată că prevalența pacienților și a simptomelor COVID-19 a fost mai mare la bărbați decât la femei. Nivelul ridicat al fumatului ar fi putut contribui la incidența înaltă de COVID-19 în rândul bărbaților. Cercetarea noastră sugerează, de asemenea, că prezența a cel puțin uneia dintre comorbidități sau a unor comorbidităților combinate sporește riscul incidenței COVID-19 și se prezintă ca un potențial factor de risc COVID-19 – rezultatele fiind asociate. Ar trebui să se depună mai multe eforturi în scopul protejării pacienților cu una sau cu mai multe comorbidități de expunerea la infecții.

## INTRODUCTION

On late December, an outbreak of a febrile respiratory illness in Wuhan city, Hubei Province, China (1) caused by the novel coronavirus (2019-nCoV) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) occurred. The World Health Organization (WHO) identified this disease as novel coronavirus disease 2019 (COVID-19) on February 11th, 2020 and declared it a pandemic on March 11th, 2020 (2). As of this writing, the total number of cases documented globally has surpassed 190 million, with over 4 million deaths (3). This fatal infection is spread mostly by large respiratory droplets produced by infected persons when coughing or sneezing, however the virus has also been found in infected people's feces and urine (4). Fever, dry cough, tiredness, nasal congestion, myalgia, sore throat, and diarrhea are the most frequent COVID-19 symptoms, whereas comorbidities include diabetes, hypertension, respiratory illness, cardiovascular disease, cancer, and others (5, 6, 7). Accordingly, the globe is taking extraordinary efforts to combat the risks presented by the developing pandemic Corona virus (COVID-19). The World Health Organization has declared the coronavirus a global health emergency. As a result, governments and people must take quick action to stop the spread of the illness and safeguard communities. In Libya, the first case diagnosed with COVID-19 (at Tripoli Medical Center) was confirmed by Public Health Reference Laboratory, National Center for Disease Control, Tripoli on March 24th, 2020. Up through December 31st, 2020, the total number of cases has risen by more than 101978 thousand and 1498 Death cases in Libya (8). The knowledge about the characteristics of novel coronavirus is limited, with only few published articles (9, 10). However, surveillance officer and the data management team have made remarkable efforts concerning COVID-19 data. The aim of this study is to determine the prevalence of COVID-19 and the impact of variables such as age, comorbidities, clinical features, the frequency of comorbidities on Gender based disparities. *The aim of the study* is to assess the Gender based disparities in the prevalence of COVID-19 confirmed cases.

## MATERIAL AND METHODS

A retrospective study was conducted on COVID-19 Laboratory confirmed cases to gain insight on

positive COVID-19 patients characteristics. Variables included the patients' demographic characteristics, comorbidities and symptoms. The completed data was collected by kobo collect toolbox, obtained from the surveillance team who agreed to participate using the kobo toolbox, covering the period from May 5 to December 2020. To display information on comorbidities, symptoms, and other categorical variables, we created frequency tables. To see whether there are any statistically significant variations in categorical variable between males and females, we used Yates-corrected chi<sup>2</sup> tests for percentages to test gender differences regarding variables. Multinomial Logistic Regression analyses were performed to evaluate the factors associated with confirmed COVID-19 cases, the results were presented as estimated odds ratio (OR) with respective 95% confidence interval (CI) and p values. A two-sided p-value <0.05 was considered statistically significant. All statistical analyses were performed using Microsoft excel and SPSS version 23 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### *Characteristics of the sample.*

A total of 6302 patients with a confirmed diagnosis of COVID-19 were identified. Of these, 2766 (44%) were females and 3536 (56.1%) were males, the mean age of our sample was 44±17.6 years. The 40-59 age group was the most representative (fig. 1), with a significant difference between men and women (p<0.05) shown in the age groups (10-79) (tab. 1).

### *Gender differences for clinical features.*

Most men and women COVID-19 confirmed patients manifested asymptomatic development (52.3%), with a corresponding sex ratio (95% CI) of 1.138 (0.025-0.074), p=0.0001 (tab. 2). Regarding the patients with one symptom upon diagnosis, namely breathing difficulties, coughing, diarrhea, high temperature, sore throat and loss of smell and taste, these were significantly more frequently found in men than women, all p<0.001, with sex ratio (95% CI) of 0.646 (0.785-1.194), whereas patients with two symptoms were also significant p<0.05, sex ratio (95% CI) of 1.117 (0.0093-0.393) and patients with three symptoms and more were significant p<0.05, with corresponding sex ratio (95% CI) of 1.134 (0.0058-0.046) (tab. 3, fig. 2).



Table 1. Number of COVID-19 cases by age group and sex.

Age group	Male (N, %) (N= 3536)	Female (N, %) (N=2766)	p-value*
0-9	38 (1.1)	47(1.7)	0.5235
10-19	185 (5.2)	162 (5.9)	0.0002
20-29	550 (15.6)	433 (15.7)	p<0.0001
30-39	839 (23.7)	523 (18.9)	p<0.0001
40-49	730 (20.6)	547 (19.8)	p<0.0001
50-59	559 (15.8)	432 (15.6)	p<0.0001
60-69	357 (10.1)	325 (11.7)	p<0.0001
70-79	192 (5.4)	164 (5.9)	0.0001
≥ 80	86 (2.4)	133 (4.8)	0.1555

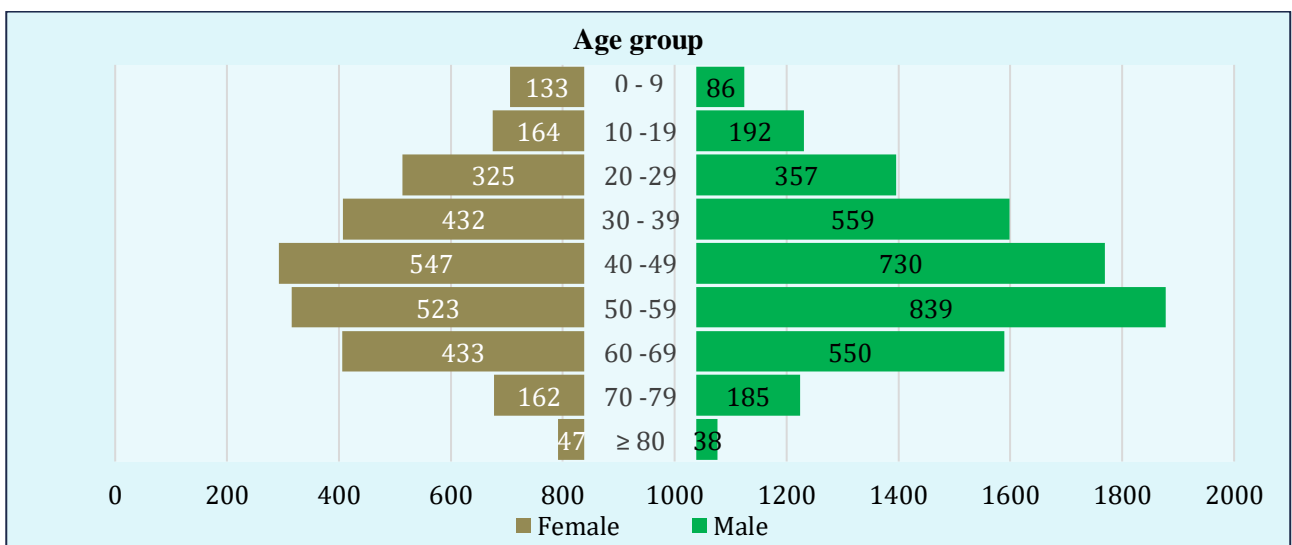


Figure 1. Age and Gender Distribution of COVID-19 patients.

Table 2. Clinical features of COVID-19 patients by gender.

Signs and Symptoms	Female (N= 2766)	Male (N= 3536)	Sex ratio	95%CI	p-value*
Patients with no symptoms	1133 (41)	1273 (36)	1.138	0.025-0.074	0.0001
1 symptom	500 (18.1)	990 (28)	0.646	0.785-1.194	p<0.0001
2 Symptoms	526 (19)	587 (17)	1.117	0.0093-0.393	0.0398
3 Symptoms or more	607 (22)	686 (19.4)	1.134	0.0058-0.046	0.0112

Table 3. Prevalence of COVID-19 symptoms.

Signs and Symptoms	Female (N=2766)	Male (N=3536)	p-value*
Patients with no symptoms	1365 (59)	1930 (55)	0.0015
Breathing difficulties	17 (0.61)	21 (0.59)	0.9149
Cough	78 (2.82)	83 (2.35)	0.2386
Diarrhea	16 (0.57)	19 (0.54)	0.873
High temperature	94 (3.4)	129 (3.6)	0.668
Sore throat	15 (0.54)	19 (0.54)	0.978
Loss of the sense of smell and taste	48 (1.73)	62 (1.75)	0.952
2 symptoms	526 (19.1)	587 (16.6)	0.009
3 or more symptoms	607 (21.9)	686 (19.4)	0.015

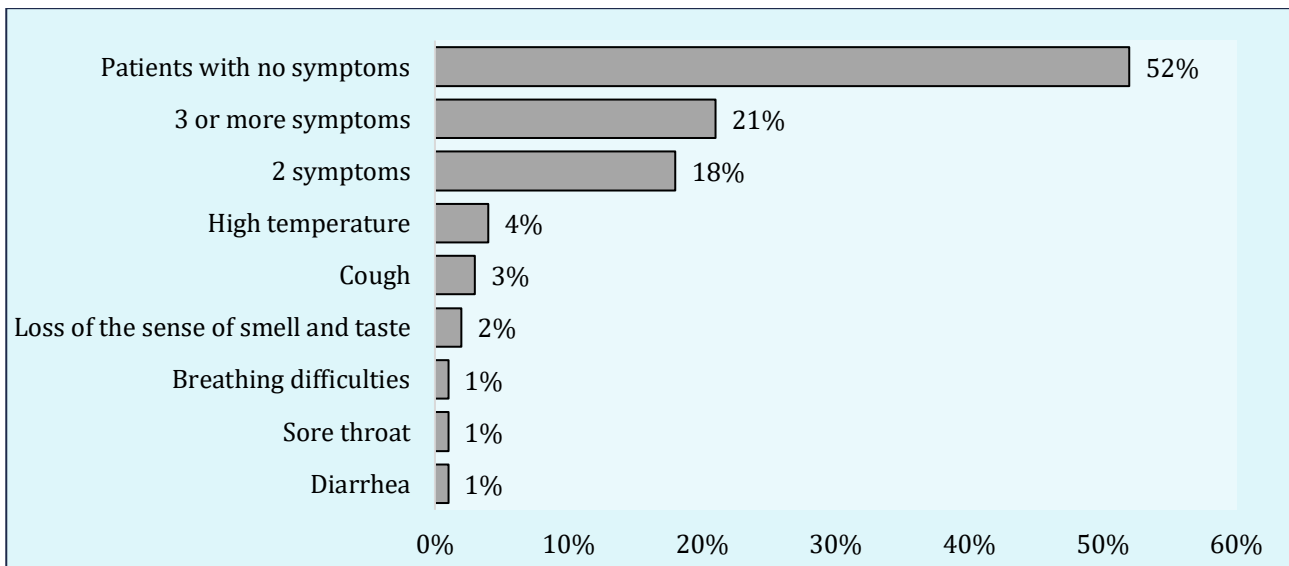


Figure 2. Prevalence of COVID-19 symptoms.

**Gender differences of comorbidity upon diagnosis.**

COVID-19 confirmed cases in patients with No comorbidity were 33%, and the percentages were comparable (35% for men and 31% for women), with a corresponding sex ratio (95% CI) of 0.886 (0.017-0.632)  $p < 0.0001$ . 12% of the patients (n=770) were diagnosed with COVID 19 and had one comorbidity, with significant difference between men to women (14% vs. 11%, respectively), with sex ratio (95% CI) of 0.786 (0.0569-0.0835)  $p < 0.0001$  (tab. 4). Furthermore, asthma, hypertension, diabetes and obesity were shown

to be significant; similarly in women to add pregnancy (tab. 5). Moreover, there was a significant difference between men to women  $p < 0.0001$  with two comorbidities in COVID-19 patients and the percentages were comparable (26.5% for men and 28% for women), with sex ratio (95% CI) of 1.057 (0.0017-0.037). Among n=1742 COVID-19 confirmed patients, those with three or more comorbidities showed a significant difference between men to women (25.1% vs. 31%, respectively), with sex ratio (95% CI) of 1.235 (0.366-0.814)  $p < 0.0001$  (tab. 6).

Table 4. Comorbidity of COVID-19 patients upon diagnosis.

Comorbidity	Female (N=2766)	Male (N=3536)	Sex ratio	95%CI	p-value*
<b>Patients with no Comorbidity</b>	854 (31)	1235 (35)	0.886	0.017-0.632	0.0008
<b>1 Comorbidity</b>	291 (11)	479 (14)	0.786	0.0569-0.0835	$p < 0.0001$
<b>2 Comorbidities</b>	765 (28)	936 (26.5)	1.057	0.0017-0.037	0.0004
<b>3 Comorbidities or more</b>	856 (31)	886 (25.1)	1.235	0.366-0.814	$p < 0.0001$

Multinomial logistic regression model of the factors predicting the contribution in the prevalence's of COVID-19 in both male and female revealed the following the predictors: age (<55, 55 to <85, >/=85), symptoms (No symptoms, 1 to 3 symptoms) and comorbidity (No comorbidity, 1 to 3 comorbidities) (tab. 6). In the gender-specific analysis, among men, (<55, 55 to <85, >/=85), no symptoms, 1- 3 symptoms, no comorbidity, more

than 3 comorbidities; asthma, hypertension, diabetes and obesity were associated with a higher rate of COVID-19 prevalence (fig. 3). Similarly, among women, age (55 to <85, >/=85), symptoms (1 to 3 symptoms) and comorbidity (No comorbidity, 1 to 3 comorbidities); asthma, hypertension, diabetes, obesity, and pregnancy were associated with a higher rate of COVID-19 prevalence (fig. 4).

Table 5. Prevalence of Comorbidity.

Risk factors and comorbidity	Female (N=2766)	Male (N=3536)	p-value*
Patients with no Comorbidity	752 (27.2)	1050 (30)	0.015
Asthma	34 (1.23)	10 (0.28)	p<0.0001
Cancer	6 (0.23)	6 (0.17)	0.6642
Cardiovascular disease	27 (0.98)	40 (1.13)	0.5647
HIV	2 (0.1)	0 (0)	0.0600
Hypertension	130 (5)	93 (2.63)	p<0.0001
Kidney disease	21 (0.76)	17 (0.5)	0.1899
Diabetes	102 (4)	185 (5.2)	0.025
Obesity	25 (0.9)	11 (0.31)	0.0020
Pregnancy	45 (1.62)	0 (0)	p<0.0001
Rheumatism	1 (0.036)	0 (0)	0.2592
Other disease (irritable bowel syndrome)	0 (0)	1 (0.028)	0.7808
Epilepsy	0 (0)	1 (0.028)	0.7808
Smoke	0 (0)	300 (8.5)	p<0.0001
2 Comorbidities	765 (28)	936 (26.5)	0.0004
3 Comorbidities or more	856 (31)	886 (25.1)	p<0.0001

Table 6. Multinomial logistic regression results for prominent character gender (n=6302).

Characteristics	Female (n=2766)				Male (n=3536)			
	B	S. E	Odd ratio [OR]	95% CI	B	S. E	Odd ratio [OR]	95% CI
<b>Age category</b>								
<55	0.199	0.076	1.220	1.051-1.416	0.310	0.030	1.364***	1.28-1.446
55 to <85	0.424	0.085	1.529***	1.294-1.806	0.085	0.051	1.089***	0.986-1.203
>/=85	0.691	0.205	1.995***	1.335-2.982	0.199	0.191	0.820***	0.564-1.191
<b>Symptoms</b>								
Patients with no symptoms	0.133	0.072	1.142	0.991-1.316	0.117	0.041	1.124*	1.037-1.217
1 symptom	-0.706	0.082	0.494***	0.420-0.580	0.683	0.055	2.025***	1.724-2.479
2 Symptoms	0.061	0.083	1.063	0.903-1.250	0.110	0.060	1.116	0.992-1.255
3 Symptoms or more	0.287	0.073	1.995*	1.335-2.992	0.122	0.056	1.130*	1.013-1.261
<b>Comorbidity</b>								
Patients with no Comorbidity	-0.360	0.074	0.691***	0.634-0.755	0.369	0.045	1.446***	1.325-1.578
1 Comorbidity	-0.422	0.093	0.608***	0.525-0.703	0.498	0.074	1.646	1.423-1.904
2 Comorbidity	0.111	0.073	0.895	0.743-0.899	0.202	0.049	1.224	1.112-1.346
3 Comorbidity or more	-0.034	0.048	1.538***	1.045-2.640	0.034	0.048	1.035***	0.942-1.137

S.E = Standard Error  
OR= odds ratios

CI= confidence interval  
\*p<.05, \*\*p<.01, \*\*\*p<.001

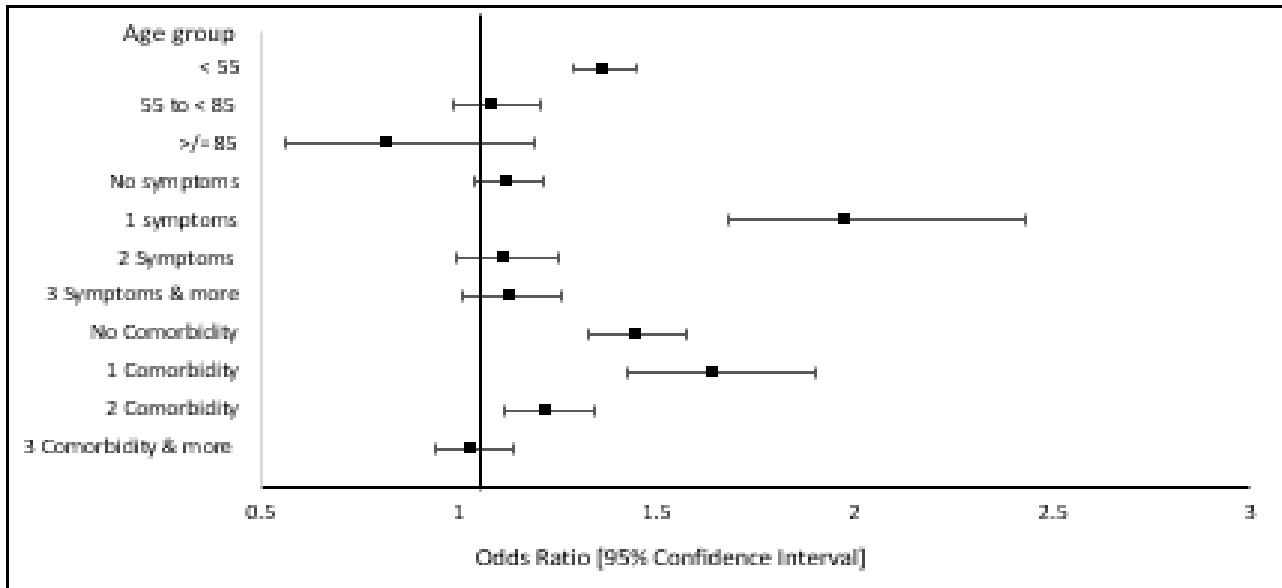


Figure 3. Forrest plot of the odds ratio and confidence intervals calculated for each variable found to be independently associated with prevalence of COVID -19 among male patients.

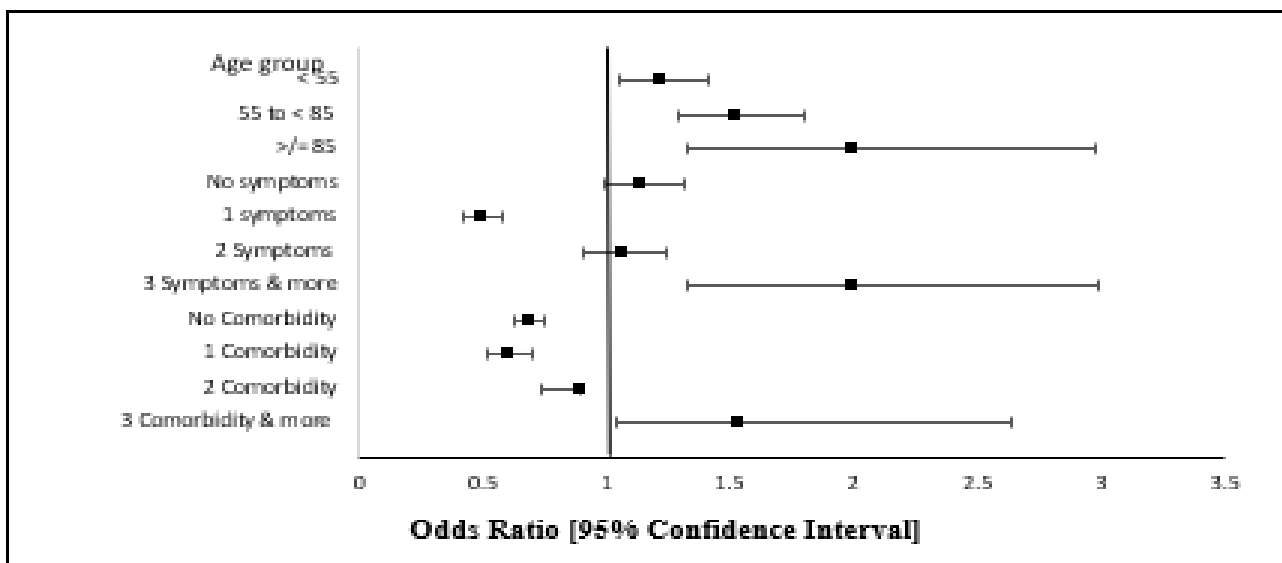


Figure 4. Forrest plot of the odds ratio and confidence intervals calculated for each variable found to be independently associated with prevalence of COVID-19 among Female patients.

**DISCUSSIONS**

Libya, like many other countries, is going through a challenging time because of the global COVID-19 issue. Furthermore, since the beginning of the war in Libya in early 2011 (11, 12, 13), the nation has experienced internal security, political, and economic crises, in addition to the looming health catastrophe in 2020. Therefore, the ability to cope with pandemic data and health information was limited.

However, according to this study, the use of a sim

ple data collection tool like the kobo gather toolbox aided the surveillance officer in collecting COVID-19 data. In previous research conducted in several countries focusing on patients’ demographics, underlying health comorbidities, socioeconomic inequities in healthcare access and quality, and environmental variables including pollution, to identify possible risk factors and susceptible groups, while other researches have looked over the impacts of these domains on COVID-19 dissemination individually, some of them haven’t considered the possibly confusing

interactions between factors. On the other hand, according to our knowledge there are limitations regarding risk factors related to COVID-19 researches in Libya. However, one study was done to explore the factors that influence in-ICU mortality rate, which revealed that risk factors such as age, BMI, laboratory findings, admission SOFA score, and quick SOFA score were strongly predictive for mortality (9). In this study, we sought to investigate the COVID-19 prevalence and their association with demographical characteristics, clinical manifestation, and comorbidities, we observed that males are more likely to be infected by COVID-19 (with 56.1%) compared to females (44%). A similar conclusion has previously been observed in another research (14, 15, 16). Men are more vulnerable than women, according to research carried out in Spain (17), because of their careless attitude about the possibility of a COVID-19 pandemic. Another Spanish study found that males and the elderly have a greater severity and case fatality rate (CFR) (18). Furthermore, females have stronger resistance, which might be attributed to female sex hormones, whereas men have lesser resistance owing to the high expression of the ACE2 receptor, which coronavirus easily attaches to (19). Other studies also showed that higher viral load in men has also been linked to ACE2 expression, reduced B cell and NK cell-specific transcripts, male hormones, and enhanced NF- $\kappa$ B inhibitor (20, 21, 22). Results showed that all age groups in both sexes were susceptible to COVID-19, showing a significant difference of  $p < 0.0001$ , however younger individuals aged between 0-19- 70+ showed lower COVID-19 incidence, while higher rate of COVID-19 cases was noticed in age groups over 20 years (aged 20-69). Furthermore this could indicate that during this study period from May to December 2020, the COVID-19 virus had the Wuhan strain, and the susceptibility to contract the virus among men or women may depend on the role of biological profile of a person; as well as their activities, were men are more likely to engage in outside activities, exposing them to conditions such as extreme weather and pollution, which might influence their response to an infection such as COVID-19. Furthermore, men's lifestyles, which include smoking, result in a high viral load and severity (23). In our study, smoking was significant  $p < 0.0001$  in male patients, thus our findings were similar to a study which suggested that those current smokers are at greater risk than

former smokers or non-smokers (24).

The prevalence of clinical symptoms among 6302 COVID-19 cases, 3896 (62%) were symptomatic and asymptomatic 38.2% and about 25% had one clinical symptom (high temperature, coughing, breathing difficulties, diarrhea, sore throat, loss of smell and taste), 18% with two symptoms and 21% with three or more symptoms. Therefore, our results on the prevalence of symptoms in adults corresponded to the results found in systemic literature review (25, 26). The findings show clearly that fever and cough are the most common symptoms, while other symptoms occur at much lower prevalence. However, there was not enough evidence on the prevalence of additional symptoms difference by age in the young and adults.

The presence of comorbidities in 67% of COVID-19 cases; with 33% had no comorbidity, 12.2% reported having at least one comorbidity, 27% had two comorbidities and 28% had reported three or more comorbidities. Furthermore, in the study the prevalence of specific comorbidities for both sexes included diabetes, hypertension, cardiovascular diseases, asthma, and obesity with  $p < 0.0001$ . In pregnant women the significant value was of  $p < 0.0001$ , thus the studies suggested that pregnancy has been identified as a risk factor for developing severe complications (27, 28). The study findings are consistent with other published studies (29, 30). However, few data were available on the frequency of combinations of comorbidities and symptoms (31, 32). The Multinomial Logistic Regression confirmed the age  $< 55$  ( $p < 0.0001$ , OR=1.364 CI95% 1.287-1.446), 3 or more symptoms ( $p < 0.0001$ , OR=1.130 CI95% 1.013-1.261) and 3 or more comorbidity ( $p < 0.0001$ , OR=1.035 CI95% 0.942-1.137) was a significant risk factor for COVID-19 prevalence in male patients, whereas women, was significant with age  $85 \geq$  ( $p < 0.0001$ , OR=1.995 (1.335-2.982), 3 or more symptoms ( $p < 0.0001$ , OR=1.995 CI95% 1.335-2.992) and 3 or more comorbidity ( $p < 0.0001$ , OR=1.538 CI95% 1.045-2.640). Our findings were similar in a meta-analysis study (25, 29, 32). Furthermore, our findings suggested that patients with comorbidities are more likely to have poorer well-being. Therefore, proper triage of patients should be implemented by carefully inquiring about their medical history; both the category and number of comorbidities should be considered because it might help iden

tify patients who would be more likely to develop adverse outcomes of COVID-19 and predicting the prognosis in patients with COVID-19. Moreover, better protection should be given to the patients with COVID-19 who had comorbidities upon confirmation of the diagnosis.

Although this study highlights the prevalence COVID-19-related symptoms, comorbidities limi-

tations must be acknowledged. The sample size was small, but most results were consistent with other studies on adults and children's patients with COVID-19. The study did not include data on the recovered COVID-19 patients. Furthermore, this study didn't evaluate the mortality rate in patients with severe COVID-19, thus a larger case series should be considered for further analysis.

## CONCLUSIONS

1. Available data indicate that the prevalence of COVID-19 patients is greater in males than in women, and that vulnerability to COVID-19 cases among young and middle-aged people is similar to that of older age groups.
2. The prevalence of COVID-19 symptoms was found to be higher in men than in women. The high prevalence of smokers might contribute to the high prevalence of COVID-19 among men.
3. Our study also suggests that the presence of at least one or combined comorbidities represent risk factors of COVID-19 prevalence and a potential risk factor for COVID-19-related outcomes. More efforts should be implemented to protect patients with one or more comorbidities from being exposed to the infection.

## CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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## FATTY ACID COMPOSITION OF DRINKING COW'S MILK TRADE NETWORKS OF KYIV

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**Keywords:** fatty acids, drinking cow's milk.

**Introduction.** There are several important aspects requiring a control of fatty acid composition in drinking milk distributed across the retail stores: milk is one of the main consumer products; fatty acids are the main energy substrates, involved in the synthesis of cellular structural components, whereas their interaction might impact the intensity of body growth and development; the fatty acid composition of milk varies depending on the diet and the animal's specific physiological state; in case of udder diseases, a change milk composition may occur; prevention of product counterfeiting distributed across the retail stores.

**Material and methods.** The present research examined milk samples from five dairy producers that distribute their products across the retailing chains in Kyiv. Milk samples were collected to determine the fatty acid composition. Milk fat was extracted following the Folch method. The milk fatty acid content assessment was carried out by gas chromatography.

**Results.** 20 fatty acids were identified in the tested milk samples. A higher level of saturated fatty acids was recorded in milk from "Ferma" and "Selyanskoye for kids" dairy producers. The highest content of unsaturated fatty acids was registered in products of "Ukrainskoe" and "Molokia" dairy trademarks.

**Conclusions.** The fatty acid composition of the drinking cow's milk distributed across trading networks in Kiev was found to be heterogeneous, by differing in the content of both long-chain saturated fatty acids and unsaturated fatty acids.

**Cuvinte cheie:** acizi grași, lapte de vacă.

**COMPOZIȚIA ACIZILOR GRAȘI ÎN LAPTELE DE VACĂ DE BĂUT DIN REȚELELE COMERCIALE DIN OR. KIEV, UCRAINA**

**Introducere.** Există câteva aspecte importante care necesită un control riguros al compoziției acizilor grași din laptele de băut distribuit în magazinele de vânzare cu amănuntul: laptele este unul dintre produsele esențiale de consum; acizii grași sunt principalele substraturi energetice implicate în sinteza componentelor structurale celulare, în timp ce interacțiunea lor ar putea afecta intensitatea dezvoltării organismului uman; compoziția acizilor grași din lapte variază în funcție de alimentație și de starea fiziologică specifică animalului; în cazul bolilor ugerului poate surveni modificarea compoziției laptelui; prevenirea contrafacerii produselor lactate distribuite în magazinele de vânzare cu amănuntul.

**Material și metode.** Prin prezenta cercetare s-au examinat mostrele de lapte de la cinci producători de lactate care își distribuie produsele prin lanțurile de vânzare cu amănuntul din or. Kiev pentru a determina compoziția acizilor grași. Grăsimea din lapte a fost extrasă prin metoda Folch. Evaluarea conținutului de acizi grași din lapte a fost efectuată prin cromatografie gazoasă.

**Rezultate.** În probele de lapte testate au fost identificați 20 de acizi grași. Un nivel mai ridicat de acizi grași saturați a fost înregistrat în laptele de la producătorii de lactate „Ferma” și „Selyanskoye pentru copii”. Cel mai mare conținut de acizi grași nesaturați a fost înregistrat în produsele mărcilor comerciale de lactate „Ukrainskoe” și „Molokia”.

**Concluzii.** Compoziția de acizi grași din laptele de vacă distribuit în rețelele comerciale din or. Kiev s-a dovedit a fi heterogenă, prin diferența în conținutul atât de acizi grași saturați cu lanț lung, cât și de acizi grași nesaturați.



## INTRODUCTION

The nutritional value of drinking cow's milk distributed by trade networks across different countries worldwide may slightly differ, regardless of the technological performance for obtaining raw milk. Certainly, the milk composition is genetically determined and varies among different species of mammals, however, significant fluctuations may also occur in the indices of drinking milk composition within the same species. If considering the lipid composition of drinking milk and disregarding the impact of pathological factors (such milk is not used), as well as product counterfeiting, then, the following should be mentioned: breed and physiological characteristics of the lactating animals; the diet composition; technologies for keeping dairy cows (grazing on pastures, exercise, transportation, etc.); technologies for obtaining drinking milk. Some researchers believe that the behaviour, exercise, and grazing intensity and duration of lactating animals can also affect the composition and quality of the milk obtained (1, 2).

As regarding certain interspecies characteristics, then eleven branched-chain fatty acids have been identified in camel milk, which are preferably C15:0, anteiso-C15:0 and C17:0 anteiso-C17:0 (3).

Cow milk lipids consist mainly of triglycerol (98%), diacylglycerol (2%), non-esterified and esterified cholesterol (<0.5%), phospholipids (1%) and free fatty acids (0.1%) (4).

A number of studies described that the digestive system of ruminants has a great impact on the quality and chemical composition of milk, as well as its suitability for cheese production. Cow's milk has a higher level of monounsaturated fatty acids and a lower n6/n3 ratio compared to sheep and goat's milk, which show a higher amount of polyunsaturated n3 fatty acids (5, 6, 7).

The milk of each dairy animal species has its own specific lipid profile used in formulating of the dairy products to obtain the intended technological and nutritional parameters. Stable milk quality and safety indices are of great importance for the food processing industry. Free fatty acids, especially the short-chain ones, show a lower taste threshold, providing characteristic organoleptic characteristics properties of fermented dairy products, particularly in cottage cheese (8, 9).

Free fatty acids also contribute to the raw milk technological suitability for being processed, as they impact on surface tension and foaming ability of the milk (10).

In mammary gland pathologies, including sub-clinical mastitis, both milk safety indicators and milk fatty acid composition may alter (11).

Drinking milk 1-2 times a month was associated with lower all-cause mortality in men compared with those who never drank milk (12). When buying milk for children, a person hopes to get a product characterized by a particular content of essential fatty acids and readily available energy, which would meet a growing body's needs, both in the structural lipid synthesis and biologically active substances, and in providing sufficient amount of ATP energy for children's growth and development (13).

The 1994 EU Council Regulation 1234/2007 and the EU Commission Regulation No 445/2007 set standards for fatty oils, including functional fats and spreads. They defined fats into different categories, such as butter, margarine and blend spreads. Spreadable fats undergo standard classification according to their fat content and whether they are dairy/non-dairy, vegetable/animal origin) (14).

Ukraine is actively implementing regulations on specific indicators providing quality and safety of food products, particularly in milk, according to the requirements of the EU. Modern requirements for raw milk involve careful analysis (15).

The following major aspects have drawn our attention to the need of monitoring the fatty acid composition of drinking milk distributed across the retail chains: milk is one of the main consumer products used by a significant category of population and its quality impacts food security within the state; fatty acids are the main energy substrate and the source of the structural lipids synthesis; milk lipids exhibit a high metabolic activity; raw milk fatty acid composition can vary depending on the animal's diet and specific physiological state; prevention of product counterfeiting.

*The purpose of this study* was to investigate the nutritional value of the lipid component of drinking milk distributed across the retail chains in Kyiv.

## MATERIAL AND METHODS

The pasteurized cow milk from five manufacturers was studied, the dairy products found in retail stores across Kyiv (Ferma, Ukrainskoe, Molokia, Zlagoda and Selyanskoe for kids). Milk samples were studied to determine the fatty acid composition.

The extraction of total lipids was performed using Folch method (16). Next, the sample preparation was carried out by hydrolysis and methylation of fatty acids. Therefore, 4 cm<sup>3</sup> of methyl sodium hydroxide solution was added to 100 mg of the obtained fat, then a reflux condenser was attached to the flask and boiled until the fat droplets disappeared, by stirring the flask content with 30-60 second intervals. 5 cm<sup>3</sup> of a methyl boron trifluoride solution was added to the flask content and boiling for up to 1 hour. 3 cm<sup>3</sup> hexane was added to the boiling mixture through the top of the reflux condenser and then removed from the heating element. 20 cm<sup>3</sup> of saturated sodium chloride solution was added to the hot mixture and stirred for 15 seconds. The upper (hexane) layer was collected for research in accordance with the current DSTU ISO standards (17). The analysis of fatty acids methyl esters was carried out via a Trace GC Ultra gas chromatograph (United States) with a flame ionization detector. The following chromatograph operating conditions were considered: column temperature was maintained at 140-240°C and detector temperature - at 260°C. A TriPlus autosampler at a dose of 1 µL was used to inject the sample into the chromatograph. The analysis duration was 65 min. Fatty acids were identified using a standard Supelco 37 Component FAME Mix sample. The quantitative spectral assessment of milk fatty acids was carried out by internal normalization and by determining their percentage content. Three parallel studies were carried out.

The statistical processing of the experimental data was carried out using the generally accepted methods. The Student's t -test was used for assessing the statistical significance of indicators. Differences between the compared indicators were considered reliable at a significance level of  $P \leq 0.05$ ,  $P \leq 0.01$ .

## RESULTS

As it has already been mentioned above, the fatty acid composition of consuming milk, distributed

across the retail chains, depends on many factors associated with both the quality of raw material supplied to the dairy processing plants and the depth of processing, namely, the technologies used by different dairy manufacturers. Therefore, the fatty acid composition of consuming milk collected from different manufacturers in Ukraine somewhat varies (tab. 1). The milk fat of all the selected milk samples collected from various trademarks included 20 fatty acids, such as: C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C14:1, C15:0, C16:0, C16:1n9c, C17:0, C17:1, C18:1n9c, C18:2n6c, C18:3n3, C21:0, C22:0, C20:3n6.

It should be noted that the studied samples met the microbiological safety criteria (lack of *Salmonella spp.*, *Listeria monocytogenes*, *Staphylococcus aureus*, coliforms) and quality standards (the number of mesophilic aerobic and facultative anaerobic microorganisms) established for milk.

Following the laboratory findings, the fatty acid composition of Ferma dairy brand (2.5% fat content) contained the highest amount of fatty acids with a chain length from C4:0 to C16:0 (59.33%) as related to the lowest fatty acids content ranging from C17:0 to C22:0, compared with the relative content of the corresponding fatty acids in selected milk samples of other dairy brands. It should be noted that the percentage of only the following saturated fatty acids C4:0, C16:0, and C18:0 showed a significant difference ( $p < 0.05$  –  $p < 0.01$ ) in the tested samples, whereas in all other cases we can only discuss about trends. This dairy brand also showed the lowest content of unsaturated fatty acids (fig. 1) (26.21%), as it decreased mainly due to C18:1n9c and C18:2n6c ( $p < 0.05$ ).

As regarding "Ukrainskoe" dairy brand (2.5% fat), it showed almost similar values to the samples of "Molokiya" (1.6% fat) and "Zlagoda" (3.2% fat) dairy brands in terms of the relative content of saturated fatty acids, the indicators ranging between 69.75% – 72.89 % (fig. 1). While the content of the total amount of saturated fatty acids in the "Selyanskoye for kids" dairy brand (2.5% fat), in terms of its indicators, was closer to the values found in milk samples of "Ferma" dairy brand. The content of unsaturated fatty acids in dairy samples of various brands increased as following: 26.19% ("Ferma") < 26.49% ("Selyanskoe for kids") < 27.11% ("Zlagoda") < 29.99% ("Ukrainskoe") < 30.25% ("Molokiya").

Table 1. Fatty acid composition of pasteurized cow's milk (M±m, %, n=5).

Fatty acids	"Ferma", 2.5% fat	"Ukrainskoe", 2.5% fat	"Molokia", 1.6% fat	"Zlagoda", 3.2% fat	"Selyanskoe for kids", 2.5% fat
<b>C4:0</b>	3.91±0.23	3.41±0.37	3.26±0.16*	2.97±0.24*	3.76±0.12
<b>C6:0</b>	2.79±0.13	2.43±0.25	2.41±0.31	2.40±0.11*	2.51±0.32
<b>C8:0</b>	1.75±0.26	1.52±0.23	1.47±0.20	1.51±0.21	1.55±0.2
<b>C10:0</b>	3.77±0.19	3.35±0.25	3.12±0.15*	3.22±0.16	3.30±0.22
<b>C11:0</b>	0.42±0.09	0.35±0.05	0.32±0.03	0.33±0.02	0.34±0.03
<b>C12:0</b>	4.11±0.21	3.76±0.39	3.50±0.17*	3.58±0.28	3.90±0.19
<b>C14:0</b>	12.12±0.30	11.48±0.31	10.96±0.36*	11.65±0.38	11.85±0.39
<b>C14:1</b>	1.63±0.04	1.59±0.25	1.45±0.04*	1.57±0.15	1.49±0.16
<b>C15:0</b>	1.52±0.11	1.31±0.13	1.22±0.12	1.36±0.14	1.37±0.15
<b>C16:0</b>	32.85±0.65	29.82±0.59*	31.08±1.11	30.76±0.59*	32.59±0.84
<b>C16:1n9c</b>	1.95±0.28	1.96±0.28	1.82±0.27	1.91±0.28	1.83±0.27
<b>C17:0</b>	0.60±0.15	0.69±0.17	0.60±0.15	0.73±0.13	0.66±0.14
<b>C17:1</b>	0.12±0.03	0.29±0.05*	0.23±0.09	0.31±0.07*	0.15±0.08
<b>C18:0</b>	9.45±0.31	10.85±0.36*	11.02±0.35*	12.23±0.46**	11.00±0.40*
<b>C18:1n9c</b>	18.76±0.57	20.82±0.71*	20.85±0.70*	20.62±0.75	19.47±0.68
<b>C18:2n6c</b>	3.04±0.25	4.45±0.30**	5.23±0.35**	3.09±0.33	3.11±0.22
<b>C18:3n3</b>	0.45±0.13	0.64±0.16	0.45±0.13	0.51±0.14	0.34±0.11
<b>C21:0</b>	0.53±0.15	1.02±0.20	0.66±0.16	1.05±0.19	0.46±0.13
<b>C22:0</b>	0.02±0.01	0.05±0.04	0.17±0.08	0.14±0.07	0.18±0.08
<b>C20:3n6</b>	0.26±0.09	0.26±0.12	0.26±0.05	0.11±0.06	0.13±0.07

\* P≤0.05, \*\* P≤0.01 – as related to data collected from Ferma dairy rademark samples (2.5% fat); these data are presented as mass fraction of fatty acids expressed in % of the fatty acid amount.

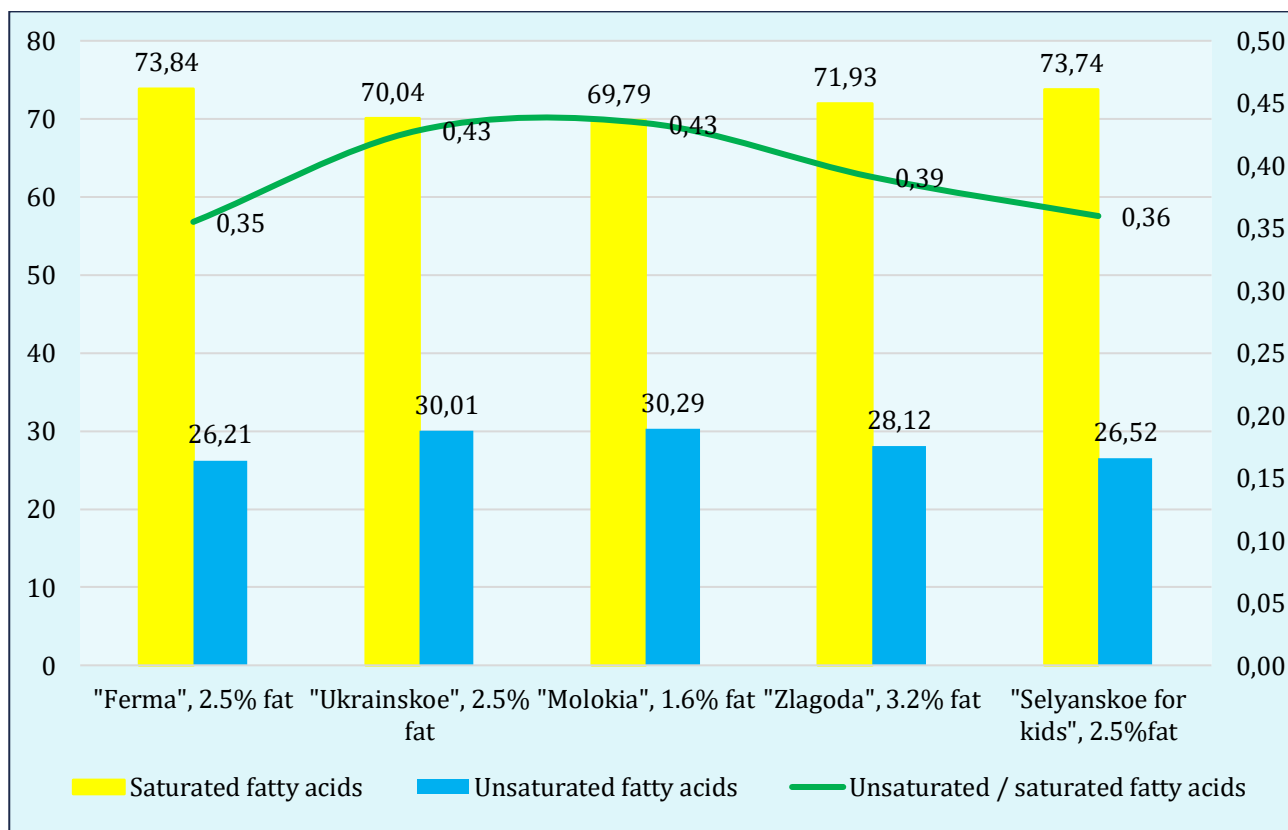


Figure 1. The ratio of the total amount of fatty acids in drinking milk.

As shown in Figure 1, the “Selyanskoe for kids” dairy brand (2.5% fat) exhibited a higher amount of saturated fatty acids. However, the high percentage was not related to chain length of the fatty acids with a from C4:0 to C12:0, as described in the samples of the “Ferma” dairy brand (2.5% fat), but rather due to the higher content of C18:0 ( $p < 0.05$ ) and C16:0 indicators.

## DISCUSSIONS

The present studies have revealed a somewhat wide range of fluctuations in the fatty acid composition of milk collected from different dairy manufacturers. There is no doubt that the fatty acids of raw milk supplied for dairy processing is decisive for the finished product characteristics, however, the impact of the processing depth of raw material on the quality of the final product should also be considered.

Certainly, under conditions when the fatty acid composition of the distributed consuming milk is not normalized, dairy producers are not significantly restricted in manipulating the nutritional value of its lipid component. Surely, there may be an increase in the content of short-chain saturated fatty acids in “Ferma” dairy brand (2.5% fat), having a certain dietary value, however, a

## CONCLUSIONS

1. The fatty acid composition of the drinking milk distributed within the trading stores in Kiev is heterogeneous and differs in the content of both low molecular weight and high molecular weight saturated fatty acids and unsaturated fatty acids. The milk samples of the “Ukrainskoe” (2.5% fat) dairy brand showed almost similar values to the samples of “Molokiya” (1.6% fat) and “Zlagoda” (3.2% fat) dairy brands, in terms of the relative content of saturated fatty acids, while according to its indicators, the content of the total amount of saturated fatty acids in the “Selyanskoye for kids” dairy brand (2.5% fat) revealed closer values to those found in “Ferma” dairy brand.

## CONFLICT OF INTERESTS

All authors declare no competing interests.

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decrease in the total amount of unsaturated fatty acids indicates a decrease in the nutritional value of the product. On the other hand, a decrease in fat content to 1.6% in “Molokiya” products do not show a negative impact on its fatty acid composition; moreover, the content of unsaturated fatty acids in these samples was the highest, which is definitely a positive factor.

The study results revealed a heterogeneous content of polyunsaturated fatty acids C18:2n6c, C18:3n3 and C20:3n6 in the samples of different studied dairy brands: the lowest content was found in the “Selyanskoe for kids”, 2.5% fat (3.58%), “Zlagoda”, 3.2% fat (3.71%) and “Ferma”, 2.5% fat (3.75%); the highest - “Ukrainskoe”, 2.5% fat (5.35%) and “Molokiya”, 1.6% fat (5.94%).

Although the Ukraine’s national system does not provide milk quality standards in terms of fatty acid composition, it should be noted that a significant increase/decrease in the content of certain fatty acids was found in milk obtained from cows with subclinical mastitis, thus indicating dairy product counterfeiting. Therefore, here arises the question regarding the amendment of the regulatory documents on controlling and preventing low-quality milk on the domestic market.

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## CORRELATION BETWEEN ECHOCARDIOGRAPHIC PARAMETERS OF LEFT VENTRICLE AND GLYCOSYLATED HEMOGLOBIN IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

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**Keywords:** children, Diabetes Mellitus, left ventricle, glycosylated hemoglobin.

**Introduction.** Deterioration of left ventricular (LV) parameters in Diabetes Mellitus (DM) can occur in the absence of other heart problems. An association between glycosylated hemoglobin (HbA1c) and changes of the LV parameters in DM has been reported. However, data regarding this association model in children with Type 1 Diabetes Mellitus (T1DM) are limited. The purpose of the work was to investigate the association between HbA1c and the LV parameters in pediatric patients.

**Material and methods.** The study was conducted on 28 children with T1DM ((aged 10-18 years, gender M (15)/F (13), duration  $\geq 5$  years). The clinical (standard medical examination) and paraclinical (biochemical dosage – HbA1c, echocardiography – LV functional and structural parameters) data was carried out. Statistical analysis used the SPSS version 20.

**Results.** The correlational study between the HbA1c and the LV parameters revealed a statistically significant positive correlation coefficient with aortic root diameter (mm) ( $r=0.7^{**}$ ,  $p<0.001$ ), left atrium (mm) ( $r=0.8^{**}$ ,  $p<0.001$ ), LV diastolic diameter (mm) ( $r=0.7^{**}$ ,  $p<0.001$ ), LV systolic diameter (mm) ( $r=0.7^{**}$ ,  $p<0.001$ ), septal wall thickness (mm) ( $r=0.5^*$ ,  $p=0.036$ ), posterior wall thickness (mm) ( $r=0.5^*$ ,  $p=0.032$ ), LV diastolic volume (ml) ( $r=0.5^*$ ,  $p=0.025$ ), LV systolic volume (ml) ( $r=0.6^{**}$ ,  $p=0.01$ ), ejection fraction (%) ( $r=0.7^{**}$ ,  $p=0.001$ ), fractional shortening (%) ( $r=0.6^{**}$ ,  $p=0.002$ ).

**Conclusions.** The results of the study show that in children with T1DM, the increase value of the HbA1c is associated with a consensual and proportional increase in the values of the parameters of the left ventricle.

**Cuvinte cheie:** copii, diabet zaharat, ventricul stâng, hemoglobină glicozilată.

### CORELAȚIA DINTRE PARAMETRII ECOCARDIOGRAFICI AI VENTRICULULUI STÂNG ȘI HEMOGLOBINA GLICOZILATĂ, LA COPIII CU DIABET ZAHARAT TIP 1

**Introducere.** Deteriorarea parametrilor ventriculului stâng (VS) în diabetul zaharat (DZ) poate exista în absența altor probleme cardiace. A fost raportată o asociere între valorile hemoglobinei glicozilate (HbA1c) și modificările parametrilor VS în DZ. Cu toate acestea, datele privind acest model de asociere la copiii cu DZ de tip 1 (DZ1) sunt limitate. Scopul lucrării rezidă în studierea asocierii dintre HbA1c și parametrii VS la pacienții pediatrici.

**Material și metode.** În studiu au fost incluși 28 de copii cu DZ1 (cu vârsta de 10-18 ani, gender M (15)/F (13), durata  $\geq 5$  ani). S-a efectuat evaluare clinică (examen medical standard) și paraclinică (dozare biochimică - HbA1c, ecocardiografie - parametrii funcționali și structurali ai VS). Analiza statistică - versiunea 20 SPSS.

**Rezultate.** Studiul corelațional dintre HbA1c și parametrii VS a relevat un coeficient de corelație pozitiv semnificativ statistic, cu diametrul aortei (mm) ( $r=0.7^{**}$ ,  $p<0,001$ ), atricul stâng (mm) ( $r=0,8^{**}$ ,  $p<0,001$ ) diametrul diastolic al VS (mm) ( $r=0,7^{**}$ ,  $p<0,001$ ), diametrul sistolic al VS (mm) ( $r=0,7^{**}$ ,  $p<0,001$ ), septul interventricular (mm) ( $r=0,5^*$ ,  $p=0,036$ ), peretele posterior al VS (mm) ( $r=0,5^*$ ,  $p=0,032$ ), volumul diastolic al VS (ml) ( $r=0,5^*$ ,  $p=0,025$ ), volumul sistolic al VS (ml) ( $r=0,6^{**}$ ,  $p=0,01$ ), fracția de ejeție (%) ( $r=0,7^{**}$ ,  $p=0,001$ ), fracția de scurtare (%) ( $r=0,6^{**}$ ,  $p=0,002$ ).

**Concluzii.** Rezultatele studiului au demonstrat că la copiii cu diabet zaharat de tip 1, creșterea valorii HbA1c este asociată cu o mărire consensuală și proporțională a valorilor parametrilor ventriculului stâng.

## INTRODUCTION

The number of children with Diabetes Mellitus (DM) is increasing every year. In populations of European origin, nearly all children with DM have T1DM, but in other populations (e.g. Japan) T2DM is more common than T1DM in this age group. It is estimated that the incidence of T1DM among children is increasing in many countries particularly in those aged less than 15 years. The overall annual increase is estimated to be around 3% with strong indications of geographic differences. Generally, 1.110.100 children younger than 20 years are estimated to have T1DM globally, and around 98.200 children under the age of 15 years are diagnosed with T1DM annually, while this estimated number increases to 128.900 when the age range extends to under 20 years (1).

Diabetes Mellitus, and the continuum of blood glucose levels even below the DM diagnostic threshold are associated with a wide range of cardiovascular conditions that collectively comprise the largest cause of both morbidity and mortality for people with DM (2). Systematic reviews indicate that the relative risk of cardiovascular diseases (CVD) is between 1.6 and 2.6%, but that the relative risk is higher among those of younger age. One of the common presentations of CVD in T1DM is deterioration of left ventricular (LV) parameters (3, 4). An association between glycosylated hemoglobin (HbA1c) and changes of the LV parameters in DM has been reported in the adult population, however, data regarding this association model in children with T1DM are limited.

*Purpose of the study:* while considering the aforementioned arguments and the impact of childhood health on further adult health, we considered choosing the following research on investigating the association between HbA1c and the LV parameters in pediatric patients with T1DM, that will contribute to the opening of new perspectives for identifying a single and effective approach, as well as for preventing cardiovascular complications of this disease to reduce the morbidity and mortality rates at a young age.

## MATERIAL AND METHODS

The study project was carried out within the PHMI Mother and Child Institute, at the Department of Pediatrics of the Pediatric endocrinology

clinic, in the city of Chisinau, the Republic of Moldova, to which 28 children with DM were admitted, aged from 10 to 17 years 11 months and 29 days, from both urban and rural areas, the patients being selected electively between November 2018 and February 2021.

*The research comprised several stages.* The 1st stage included 28 children with DM, who were selected based on the inclusion/exclusion criteria, and made up the research group by the following criteria: the age of 10-17 years 11 months and 29 days (inclusive); with T1DM and received insulin therapy only; the child's parent or guardian consent, as well as children's assent (age  $\geq 14$  years) on research participation; being a citizen of the Republic of Moldova; ability to effectively communicate with the researcher; ability to understand and follow the study requirements; sufficient understanding in signing the informed agreement and written assent.

The study exclusion criteria for the patients were the following: T2DM, hypertension, cardiomyopathy, valvular and congenital heart disease, having a suggestive clinical examination, confirmed by specialized examinations; acute conditions, whether or not accompanied by fever, whether or not undergoing treatment, chronic respiratory, cardiovascular, gastrointestinal, renal, neurological, endocrine, etc., disorders, whether or not undergoing treatment; the child's parents or legal representative disagreement, child's refusal to participate in the research, low compliance, patient's refusal to be included in the study.

In the 2nd stage the participants underwent an examination, which included the clinical (standard medical examination) and paraclinical (biochemical dosage – HbA1c, echocardiography – LV functional and structural parameters) data.

The 3rd stage included a statistical analysis of the obtained results. Practical conclusions and recommendations, based on the obtained results, were traced out in the 4th stage of the study.

All the participants were selected and informed about the research stages, being enrolled only based on personal agreement, following a detailed explanation on the requirements and procedures of necessary investigations by discussing with each subject individually. All the procedures were performed, based on children's parent and legal representative consent, as well as

on written assent of children  $\geq 14$  years old. They were not paid and have not suffered any financial costs for participation.

**Anthropometry.** Weight was measured using weighing scales to the nearest 0.1 kg. Height was measured using a stadiometer and was expressed in centimeters with no decimal. Body mass index (BMI) was calculated as weight divided by the square of height ( $\text{kg}/\text{m}^2$ ). BMI was categorized according to the Centers for Disease Control and Prevention (CDC) age- and sex-specific growth charts. The following categories were used: underweight: <5th percentile, normal weight: 5th to 85th percentile, overweight: 85th to 95th percentile, and obese: >95th percentile, and BMI ranging from -2 standard deviations (SD) to less or equal to +1 SD (which corresponds to BMI  $25 \text{ kg}/\text{m}^2$  at 19 years); overweight was defined as BMI less than +2 SD (which corresponds to BMI  $30 \text{ kg}/\text{m}^2$  at 19 years), obese as BMI >+2 SD, and underweight as <-2 SD (5). Body surface area (BSA, Dubois formula) =  $0.20247 \times \text{height (m)}^{0.725} \times \text{weight (kg)}^{0.425}$  (6).

**Blood pressure (BP).** Points that were taken into account during the measurement: before measuring BP, the child should be placed in a comfortable position for 3-5 minutes. The measurement had to be performed in the right arm and at the level of the heart. The height of the extended part of the cuff should cover 80-100% of the circumference of the arm, and the width – at least 40% of the circumference of the arm. The lower end of the cuff should be placed 2-3 cm above the antecubital fossa, and the stethoscope should be placed on the brachial artery. BP was estimated with an automatic electronic sphygmomanometer (Omron M7 Intelli IT, Vietnam). BP was determined through systolic blood pressure (SBP) and diastolic blood pressure (DBP) readings in mmHg. The measurement was made three times, with a five-minute interval between measurements, using the average of the last two. The procedure was carried out following the recommendations of the American Academy of Pediatrics (AAP) and European Society of Hypertension (ESH). The classification was made following the criteria proposed by the AAP and ESH, which establishes the percentiles of SBP and DBP for sex, age, and size. According to AAP, and ESH guidelines, a BP value below the 90th percentile by age, sex, and height is considered as normal BP. Arterial Hypertension (AH) is defined as a

systolic and/or diastolic blood pressure measured clinically at or above the 95th percentile (7, 8).

The high blood pressure in adults guideline (American Heart Association and American College of Cardiology) is recommended to be used for individuals aged 13 years and older by the AAP guideline and for individuals aged 16 years and above by the ESH guideline (9).

**Laboratory analyses.** HbA1c was performed in the Mother and Child Institute's laboratory. The patients were classified according to ISPAD 2018 (International Society for Pediatric and Adolescent DM) guidelines which consider HbA1c < 7.5% to be the level for optimal control (10).

**Insulin dosage.** The insulin requirement was calculated according to standard protocol (1 U/kg/day) (11).

**Cardiovascular evaluation.** According to the American Society of Echocardiography (ASE) pediatric guidelines (12), two-dimensional guided M-mode echocardiograms were obtained from all children with T1DM. The data of interest in echocardiography (EcoCG) were left ventricle (LV) systolic and diastolic function parameters, LV mass index (LVMI), left ventricular geometry, and aortic root measurements. Left ventricular ejection fraction (EF) and shortening fraction (SF) were used as measurements of LV systolic function; EF was calculated using the biplane Simpson method in the apical views of the heart as recommended by the ASE. SF was calculated in the parasternal short-axis views using M-mode data. The other M-mode measurements calculated include interventricular septal thickness in diastole, LV end-diastolic dimension, the posterior wall thickness at the end diastole, and LV end-systolic dimension. The relative wall thickness and indexed LV mass was also calculated using the above M-mode measurements. RWT was calculated using the formula  $(2 \times \text{PWd})/\text{LVEDD}$ . The LV mass was calculated using the Devereux formula (13). The LVMI was then calculated by dividing the calculated LV mass in grams by the patient's height (in meters) raised to the power of 2.7 ( $\text{LVMI} = \text{left ventricular mass (g)}/\text{height (m)}^{2.7}$ ).

The diameter of the aortic root was calculated in the parasternal long-axis views during systole. It represents the maximal diameter of the aorta at the level of the sinuses of Valsalva (12).



*Definitions of EcoCG data.* LVH is defined as LVMI of greater than 95th percentile for age and gender (14). Using LVMI and RWT, LV geometry is generally classified into four patterns: normal geometry: normal LVMI and RWT; concentric remodeling: normal LVMI and increased RWT; eccentric LV hypertrophy: increased LVMI and normal RWT; and concentric LV hypertrophy: increased LVMI and RWT (15).

*Z-scores of cardiac structures/Detroit data.* Calculate the z-scores of cardiac structures (EcoCG) was related to body surface area (6).

*Ethics.* The study complied with the international standards of medical ethics, developed by the Declaration of Helsinki, regarding confidentiality and personal data protection of the participants. The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (report no. 42 of 17.06.2019). The resulting data were revealed only to the concerned participant, the personal data of each subject were not used and will not be used for any other purpose. The research was conducted according to the principles of the Declaration of Helsinki.

*Statistics.* The data collected from the primary material were introduced in the electronic database. Data were analyzed using the Statistical Package for the Social Sciences program (IBM-

SPSS) version 20. Descriptive statistics presented as frequencies, proportions (%), mean and standard deviation according to the variable type. Level of significance was set at  $\leq 0.05$  to be significant difference or correlation.

## RESULTS

*Characteristics.* There were 28 children with T1DM (duration of T1DM  $\geq 5$  years; absence of AH, insulin therapy) enrolled in the study, the mean age of the patients was  $13.7 \pm 2.35$  (male patients were 15 (56.7%), female 13 (43.3%)).

*The results of the selective analysis of anthropometric, hemodynamic and biochemical parameters.*

The studied group was characterized by the following values (tab. 1): weight (kg) =  $53.0 \pm 17.0$  (according to the percentiles - 5<sup>th</sup> to <85<sup>th</sup>), height (cm) =  $157.2 \pm 36.7$  (according to the percentiles - 5<sup>th</sup> to <85<sup>th</sup>), body mass index (kg/m<sup>2</sup>) =  $19.0 \pm 4.5$  (according to the percentiles - 5<sup>th</sup> to <85<sup>th</sup>, and according to the Z score =  $-2 \geq z\text{-score} < +1$ ), and body surface area (cm<sup>2</sup>) =  $1.52 \pm 0.3$ . Systolic blood pressure (mm Hg) =  $115.7 \pm 12.3$  (according to the percentiles <90<sup>th</sup>), diastolic blood pressure (mm Hg) =  $75.2 \pm 8.7$  (according to the percentiles - <90<sup>th</sup>), HbA1c (%) =  $9.2 \pm 2.4$  (females > males, aged >15 years old).

Table 1. The values of selective anthropometric, hemodynamic and biochemical parameters in children included within the research.

Variable	Total (n=28)
Gender (M/F)	15/13
Age, M $\pm$ m, (years)	13.7 $\pm$ 2.35
Duration of T1DM, M $\pm$ m, (years)	6.51 $\pm$ 3.2
Height, M $\pm$ m, (cm)	157.2 $\pm$ 36.7
Weight, M $\pm$ m, (kg)	53.0 $\pm$ 17.0
BMI, M $\pm$ m, (kg/m <sup>2</sup> )	19.0 $\pm$ 4.5
BSA, M $\pm$ m, (cm <sup>2</sup> )	1.52 $\pm$ 0.3
Systolic blood pressure, M $\pm$ m, (mm Hg)	115.7 $\pm$ 12.3
Diastolic blood pressure, M $\pm$ m, (mm Hg)	75.2 $\pm$ 8.7
HbA1c, M $\pm$ m, (%)	9.2 $\pm$ 2.4

Note: values are presented as mean  $\pm$  standard deviation for a number of values; BMI - body mass index; BSA - body surface area; HbA1c - hemoglobin A1c.

*The results of the selective analysis of echocardiographic parameters of left ventricle.* The studied group was characterized by the following values (tab. 2): aortic root diameter (mm) =  $24.5 \pm 6.0$ , left atrium (mm) =  $26.8 \pm 6.2$ , right atrium<sup>1,2</sup> (mm) =  $29.0 \pm 7.3/30.1 \pm 7.2$ , right ventricle (mm)

=  $15.1 \pm 4.0$ , LV diastolic diameter (mm) =  $41.3 \pm 9.6$ , LV systolic diameter (mm) =  $25.4 \pm 5.9$ , interventricular septal thickness at end-diastole (mm) =  $7.5 \pm 1.9$ , posterior wall thickness at end-diastole (mm) =  $7.4 \pm 1.8$ , LV diastolic volume (ml) =  $81.9 \pm 24.6$ , LV systolic volume (ml) =

25.2±7.7, ejection fraction (%) = 65.7±14.8, fractional shortening (%) = 36.7±8.4, LV Mass (g) = 104.1±21.6, LV Mass Index (g/m<sup>2</sup>) = 27.06±4.4 (and with right atrium<sup>1,2</sup> (mm) (r=0.6\*\*, p=0.003), right ventricle (mm) (r=0.6\*\*, p=0.003), (height (cm) (r=0.7\*\*, p<0.001), weight (kg) (r=0.5\*, p<0.5), body mass index (kg/m<sup>2</sup>) (r=0.5\*, p<0.5), systolic BP and diastolic BP (mm Hg)). Diastolic function of LV has not been studied, being considered a research limitation.

Z-scores of cardiac structures/Detroit data. Aortic root diameter = +3.0, left atrium (mm) = +0.62,

right ventricle = -1.31, LV diastolic diameter = -0.75, LV systolic diameter = -0.67, interventricular septal thickness at end-diastole = +0.24, posterior wall thickness at end-diastole = +0.63, LV Mass (g) = -0.59.

The results of the evaluation of the types of remodeling of the left ventricular myocardium. The types of pathological remodeling were distributed as follows: 5.0% (n=2) – concentric LV hypertrophy, 5.0% (n=2) – concentric LV remodeling and 5.0% (n=2) – eccentric LV hypertrophy ((85.0% (n=22) participants showed a normal LV geometry pattern).

Table 2. The values of selective EcoCG parameters, in particular parameters for LV myocardial remodeling in children included within the research.

Variables	Total (n=28)
LA (mm)	26.8±6.2
RA1 (mm)	29.0±7.3
RA2 (mm)	30.1±7.2
RV (mm)	15.1±4.0
RWT (mm)	7.5±1.9
IVSd (mm)	7.5±1.9
LV Mass (g)	104.1±21.6
LV Mass Index (g/m <sup>2</sup> )	27.06±4.4
PWd (mm)	7.4±1.8,
LVEDD (mm)	41.3±9.6,
LVESD (mm)	25.4±5.9
LVEDV (ml) 3b,	81.9±24.6
LVESV (ml)	25.2±7.7
LVFS (%)	36.7±8.4
LVEF (%)	65.7±14.8

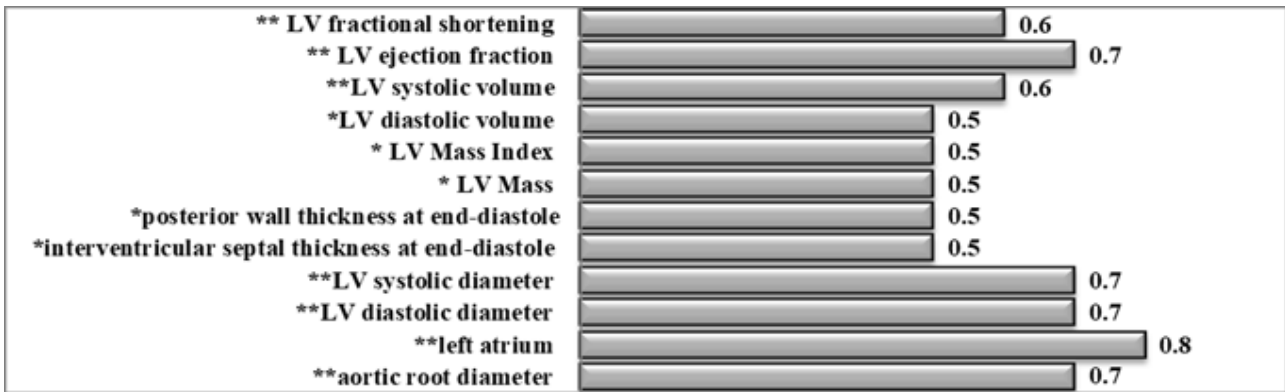
Note: values are presented as mean ± standard deviation for numerical data; LA – left atrium; RA – right atrium; IVSd – interventricular septal thickness at end-diastole; PWd – posterior wall thickness at end-diastole; LVEDD – left ventricular end - diastolic diameter; LV Mass – left ventricular mass; RWT – left ventricular relative posterior wall thickness; LVESD – left ventricular end - systolic diameter; LVEDV – left ventricular end - diastolic volume; LVESV – left ventricular end - systolic volume; LVMI – LV Mass Indexed to Body Surface Area; LVEF – left ventricular ejection fraction; LVFS – LV fractional shortening.

Correlation between selective EcoCG parameters of LV and glycosylated hemoglobin (fig. 1). The correlational study between the HbA1c and the LV parameters revealed a statistically significant positive correlation coefficient with aortic root diameter (mm) (r=0.7\*\*, p<0.001), left atrium (mm) (r=0.8\*\*, p<0.001), LV diastolic diameter (mm) (r=0.7\*\*, p<0.001), LV systolic diameter (mm) (r=0.7\*\*, p<0.001), interventricular septal thickness at end-diastole (mm) (r=0.5\*, p=0.036), posterior wall thickness at end-diastole (mm) (r=0.5\*, p=0.032), LV Mass (g) (r=0.5\*, p=0.038), LV Mass Index (r=0.5\*, p=0.038), LV diastolic volume (ml) (r=0.5\*,

p=0.025), LV systolic volume (ml) (r=0.6\*\*, p=0.01), LV ejection fraction (%) (r=0.7\*\*, p=0.001), LV fractional shortening (%) (r=0.6\*\*, p=0.002) (and, also, with right atrium<sup>1,2</sup> (mm) (r=0.6\*\*, p=0.003), right ventricle (mm) (r=0.6\*\*, p=0.003), (height (cm) (r=0.7\*\*, p<0.001), weight (kg) (r=0.5\*, p<0.5), body mass index (kg/m<sup>2</sup>) (r=0.5\*, p<0.5), systolic blood pressure and diastolic blood pressure (mm Hg)).

## DISCUSSIONS

The main cause of death in European countries is cardiovascular diseases (CVD). CVDs tend to pre



Note: \*\* p<0,001; \* p<0,05.

Figure 1. Statistical correlations of HbA1c with left ventricular (LV) parameters.

sent at a younger age in patients with DM than in the general population (16). The SEARCH for Diabetes in Youth Study showed that significant complications severely affect the quality of life of DM early in their life (17). Therefore, adolescence and young adulthood are the best periods for actions to lower cardiovascular risks.

Studies on adults with T1DM were reported an association between HbA1c and changes of the LV parameters (the individuals with DM have increased LV wall thickness and mass, and impaired diastolic function in the absence of overt LV systolic dysfunction) (18). However, data regarding this association model in children with T1DM are limited.

Presumptively, the duration of DM is closely associated with the onset and severity of cardiovascular complications in T1DM (18). The duration of the disease is probably the strongest predictor for the development and progression of diabetic cardiomyopathy (structural and functional changes, in our study – was confirmed pathological LV remodeling patterns, respectively, concentric LV hypertrophy (5.0%), concentric LV remodeling (5.0%), and eccentric LV hypertrophy (5.0%), systolic function – no change and diastolic function – was not performed). Besides DM duration, the magnitude of hyperglycemia is also a strong risk factor for the development and progression of cardiovascular complications.

The Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications study showed that the progression of complications can be reduced by strict glycemic control (19). HbA1c levels less than 7.5% are recommended to reduce future complications according to the ISPAD 2018 (10). In our study, we reported a mean HbA1c of 9.2% (most of the participants were not achieving the

target control, similar in other researches – Ogugua C. F. et al. – 11.4% (20), Aljuhani F. et al. (9.7%) (21), Stankute I. et al. – 8.5% (22)). We found that young females have worse glycemic control than males, which is consistent with other studies' results (23). Of all age groups, patients aged 15 years old have the worst glycemic control, probably because of adjustments in the endocrine system, and increased independence in DM care during adolescence makes achieving optimal HbA1c really difficult (24).

During the research, it was performed the estimation of the correlation between HbA1c and selective EcoCG parameters of LV, in particular with LV diastolic and systolic diameter, interventricular septal thickness at end-diastole, the posterior wall thickness at end-diastole, LV Mass, LV Mass Index, LV diastolic and systolic volume. The results showed that in children with T1DM, the increased value of the HbA1c is associated with a consensual and proportional increase in the values of the parameters of the LV (p<0.05). In other researches, for example, in the study conducted by Bagheri M. et al. the effect of HbA1c level on increase, LV mass was not significant, only some demographic factors (age, height, weight, maximum BP) had positive and meaningful relationships with an LV posterior wall (p<0.05) (25), but, Seferovic J. et al. showed that the LVMI was associated with higher fasting glucose and HbA1C, (indicating the possible role of hyperglycemia in LV mass increase) (26).

Chronic increase in plasma glucose level is associated with LV mass increment. Saad I. A. et al. showed that improved glycemic control in patients with T1DM is associated with regression of septal thickness and LV mass without significant effect on systolic or diastolic function (27). Also, Weinrauch L. A. et al., in a study involving

patients with T1DM showed improvement in measures of heart rate variation correlated with a decrease in LV mass and dimensions after 12 months follow-up and this paralleled glycemic control (28).

Increased LVMI could be a potential, pre-symptomatic marker of myocardial structural change in T1DM. Also, LVMI was associated with higher HbA1c, indicating the possible role of hyperglycemia in LV mass increase (26). In our study, LV ejection and shortening fraction were normal, and we found an association with HbA1c levels ( $r=0.7^{**}$ ,  $p=0.001$ ;  $r=0.6^{**}$ ,  $p=0.002$ ). Indeed, the association between LV systolic function and dysglycemia has been inconsistent across previous studies. The Cardiovascular Health Study showed no association between the severity of DM and LV fractional shortening. An early analysis from the Framingham Heart Study showed a slight decrease in fractional shortening only among males (adult patients) (29). In a cardiac MRI study of 1603 Framingham Study Offspring participants, there was no association between DM and MRI-derived LV ejection fraction) (30).

On the other hand, Yilmaz S. et al. found that duration of DM had a positive significant correlation with LA ( $r=0.6$ ,  $p=0.0001$ ), Ao ( $r=0.3$ ,  $p=0.05$ ), PA ( $r=0.5$ ,  $p=0.0001$ ), IVS ( $r=0.3$ ,  $p=0.04$ ), LVEDD ( $r=0.4$ ,  $p=0.03$ ), LVESD ( $r=0.4$ ,  $p=0.02$ ) and SV ( $r=0.4$ ,  $p=0.03$ ) measured at the first time. On the other hand, no significant correlations were found between EcoCG measurements and HbA1c or insulin dose (duration of DM was significantly higher in patients with LV hypertrophy (LVH), and, though there was a trend for patients with LVH to have the level of HbA1c to be higher ( $9.5\pm 0.8\%$ ) than those with-

out LVH ( $8.5\pm 1.5\%$ ), this trend was not significant statistically) (31). Other authors reported that there is no correlation between HbA1c and the development of cardiovascular changes in children and adolescents with T1DM (31). On the other hand, Stakos D. A. et al. stated that T1DM is associated with cardiovascular abnormalities and early detection and treatment of these abnormalities may help to prevent the natural progression of the disease (32).

The seemingly contradictory results of various studies on the effect of glycemic control can be partly explained by the statement that diabetic cardiomyopathy appears to consist of two major components: the first being a short-term physiological adaptation to metabolic changes and could be reversible, probably can be cataloged and in the case of children in our research, because the second is degenerative changes for which the myocardium has a limited ability to repair (27).

*Several limitations of the current study must be taken into account.* The current study included a relatively small number of patients, although the T1DM is considered to have a high incidence rate. Another limitation was the lack of diastolic function assessment, which is a significant predictor of CVD risk.

Finally, the findings of our study highlighted that the management of T1DM should be multifaceted and most importantly include the glycemic control, and the EcoCG examination should be recommended to assess the presence of modifications of LV myocardium (structural and functional), which will allow detecting children with DM, who are at higher risk for developing cardiovascular complications.

## CONCLUSIONS

1. We concluded that good glycemic control in children with T1DMs could improve some structural and functional parameters of the heart while failure to achieve glycemic control leads to their deterioration.
2. Follow up and early detection of myocardial structural and functional changes in young patients with T1DM contribute to better knowledge of diabetic cardiomyopathy and may help to prevent the natural progression of the disease.
3. We recommend that close observation should begin early and should include detection of cardiac alterations, as well as other diabetic complications.

**CONFLICT OF INTERESTS**

The authors do not declare any conflict of interest.

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## MAMMAL FAUNA OF CHISINAU AIRPORT, REPUBLIC OF MOLDOVA

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**Keywords:** Chisinau airport, mammals, rodents, abundance, fox, trophic connections.

**Introduction.** Airport territories are large, containing a high variety of biotopes, mostly open type, and are relatively protected against intense human activity, thus creating favorable conditions for many mammal species that serve as trophic source for many bird species.

**Material and methods.** The studies were performed during 2012-2014 on the territory of Chisinau airport and within the adjacent ecosystems. The mammals were recorded by direct observations, based on traces and trophic activity on routes ranging from 1 to 3 km. The small mammals were assessed with traps. The bat species were identified according to their flight pattern and using the ultrasound detector.

**Results.** In the airport, 31 species of mammals were registered: 5 insectivore species, 7 bat species, 14 rodent species, 1 hare species and 4 carnivorous species. The most widespread, common and abundant were the rodents and the fox. Among small rodent species, the most abundant was *Apodemus sylvaticus* with 51.6%, followed by the *Microtus arvalis* (38.9%), while on the airport grassland, the field vole dominated with more than 60%. The diet of *Athene noctua* included predominantly *Mus musculus* with over 51%, followed by *M. arvalis* with 23.7%. 7 species are rare and 5 protected – bicolor shrew and 4 bat species.

**Conclusions.** The airport territory and adjacent ecosystems provide favorable conditions for many mammal species. The presence of rodents favors the occurrence of rather high number of prey birds, which represent a threat for the safety of aircraft flights. The fox can pose direct threat to flight safety.

**Cuvinte cheie:** aeroportul Chişinău, mamifere, rozătoare, abundenţă, vulpe, conexiuni trofice.

### FAUNA DE MAMIFERE DIN AEROPORTUL CHIŞINĂU, REPUBLICA MOLDOVA

**Introducere.** Teritoriile aeroporturilor sunt extinse, conţin multe biotopuri, majoritatea de tip deschis, fiind relativ protejate de activităţile umane intense, creând astfel condiţii favorabile pentru mamiferele care pot servi drept sursă trofică pentru păsările de pradă.

**Material şi metode.** Cercetările au fost efectuate în 2012-2014, pe teritoriul aeroportului Chişinău şi în ecosistemele adiacente. Mamiferele au fost înregistrate prin observaţii directe, după urme şi activitate trofică, pe trasee de 1-3 km. Mamiferele mici au fost evaluate cu ajutorul capcanelor, iar lilieci au fost identificaţi după particularităţile de zbor şi cu detectorul de ultrasunete.

**Rezultate.** În aeroport au fost înregistrate 31 specii de mamifere: 5 specii insectivore, 7 specii de lilieci, 14 specii de rozătoare, 1 specie de iepuri şi 4 specii de carnivore. Cele mai răspândite şi prolifică au fost rozătoarele şi vulpea. Dintre rozătoare, cea mai frecvent atestată a fost *Apodemus sylvaticus* – 51,6%, urmată de *Microtus arvalis* (38,9%), iar pe pajiştea din aeroport a dominat şoarecele de câmp cu peste 60%. În raţia *Athene noctua* dominant a fost *Mus musculus*, cu peste 51%, urmat de *M. arvalis* cu 23,7%. Au fost semnalate 7 specii rare şi 5 specii protejate – chiţcanul de câmp şi 4 specii de lilieci.

**Concluzii.** Teritoriul aeroportului şi ecosistemele adiacente oferă condiţii favorabile pentru multe specii de mamifere. Prezenţa rozătoarelor favorizează atragerea păsărilor de pradă, care reprezintă o ameninţare pentru siguranţa zborurilor. Vulpea, la rândul ei, poate periclita siguranţa zborurilor.

**INTRODUCTION**

The territories of the airports are spacious, including many different biotopes, mainly of open type, and are protected from visiting by people, thus creating favorable conditions for the existence of a large number of bird species, as well as mammals, reptiles, amphibians, and insects that serve as trophic source for many terrestrial vertebrate species. The animals are attracted by the abundance of food resources, low anxiety factor, the availability of places for food, rest, shelter and breeding. The presence of vertebrate species, especially of birds, on the airport territories can cause serious problems to aviation. Wildlife aircraft collisions cause losses of human lives and financial losses for the aviation industry (1).

Although bird species are the main risk factor for aircraft safety, many other terrestrial vertebrate species can present direct or indirect threat to aviation. The large and medium-sized mammal species are a potential risk for aircraft flights, such as deer, red deer, fox, coyote, hare (2, 3, 4). The rodent species have an indirect impact, being the main prey for many vertebrate predator species. In many parts of the world, regulating the number of mammals, especially rodents at airports, is a serious problem (5-8).

*The purpose of the study* was to assess the mammal fauna in Chisinau airport and adjacent territories in order to reveal its diversity and the species that can have a direct or indirect impact upon aircraft flight, as well as the rare species occurring in the area.

**MATERIAL AND METHODS**

The Chisinau airport is situated at the altitude of 122 m, with coordinates 46°55'40"N 28°55'51"E in the eastern part of the city, extending on a surface of 4271 m<sup>2</sup>. The studies were performed during 2012-2014 on the territory of Chisinau airport and adjacent ecosystems. Within the airport, biotopes are represented by grasslands (mowed and unmowed), sectors with shrub vegetation and sectors with different types of technical buildings, including abandoned ones, tree vegetation near the buildings and several small water basins for technical purpose (fig.1). The adjacent biotopes are represented by various agroecosystems (orchards, vineyards, corn, sunflower and cereals), private gardens, sectors with buildings, forest belt, grasslands, fallow ground, wet habitats, as well as ecotones that create convenient transition zones to the airport many vertebrate animal species.



Figure 1. Open type biotopes in Chisinau airport.

The mammals were recorded by direct observations, according to the traces and trophic activity (carnivorous mammals) on routes ranging from 1 to 3 km. The small mammals were assessed with traps; 7,000 trap-nights were used and more than 300 animals were caught. The density of subterranean mammal species (mole and mole rat) was determined by direct observations and by count-

ing the molehills. The density of the hedgehog was determined by direct observation during activity hours and by the presence of trophic remains. The registration of bats was carried out in the evening by identifying species after the flight pattern and using the ultrasound detector.

The density of medium-sized mammals was assessed as individuals per hectare. In the commu-



nities of bats and small mammals (shrews and rodents) the relative abundance of each species was determined.

Pellets of the little owl (*Athene noctua*) were collected from an abandoned building situated in the central part of the airport. Each pellet was measured, weighed and afterwards unfolded. The bone fragments were cleaned and sorted into categories. Small mammal species were determined according to cranial bones and dentition (9, 10).

## RESULTS

The mammal fauna of Chisinau airport was represented by 31 species – 5 insectivore, 7 bat, 14 rodent, 1 lagomorph, 4 carnivorous species (tab. 1).

Among insectivorous mammals, represented by 5

species, 3 species were found both on the airport territory and within the adjacent ecosystems. The white-breasted hedgehog was observed in the spring-autumn period in the evening hours with a density of 0.3-1 ind./ha in the airport and about 1-2 ind./ha in adjacent biotopes. Mole density varied from 1 to 2 ind./ha and reached 4 ind./ha in optimal adjacent habitats.

The lesser white-toothed shrew (*Crocidura suaveolens*) is the most anthropophilous species among shrews and was often found in most ecosystems, including buildings, whose abundance constituted about 2% of the community of small mammals. The common and bicolor white-toothed shrews were found only in wet biotopes adjacent to the airport (banks of ponds, rivers, swampy habitats) with an abundance of 0.7-1%.

Table 1. Fauna of mammals on Chisinau airport and adjacent biotopes.

No	Species	Density/abundance		Status
		Airport territory	Adjacent biotopes	
<b>Mammalia</b>				
1.	<i>Erinaceus roumanicus</i>	1 ind./ha	2 ind./ha	Common
2.	<i>Talpa europaea</i>	1-2 ind./ha	2-4 ind./ha	Common
3.	<i>Sorex araneus</i>	-	0.7%	Rare
4.	<i>Crocidura leucodon</i>	-	1.0%	VU
5.	<i>Crocidura suaveolens</i>	0.8%	1.2%	Common
6.	<i>Myotis daubentonii</i>	-	9.3%	VU
7.	<i>Myotis mystacinus</i>	1.2%	3.1%	VU
8.	<i>Nyctalus noctula</i>	45.9%	38.2%	Common
9.	<i>Pipistrellus pygmaeus</i>	17.6%	12.8%	Common
10.	<i>Eptesicus serotinus</i>	32.7%	26.7%	Common
11.	<i>Vespertilio murinus</i>	-	3.8%	EN
12.	<i>Plecotus austriacus</i>	2.6%	6.1%	VU
13.	<i>Nannospalax leucodon</i>	1-2 ind./ha	2-4 ind./ha	Common
14.	<i>Muscardinus avellanarius</i>	-	0-2 ind./ha	Rare
15.	<i>Sciurus vulgaris</i>	1 ind./ha	2-3 ind./ha	Common
16.	<i>Arvicola terrestris</i>	-	2-10 ind./ha	Rare
17.	<i>Rattus norvegicus</i>	1 ind./ha	1-2 ind./ha	Common
18.	<i>Mus musculus</i>	2.9%	24.7%	Abundant
19.	<i>Mus spicilegus</i>	1.9%	8.4%	Common
20.	<i>Apodemus sylvaticus</i>	51.6%	40.2%	Abundant
21.	<i>Apodemus agrarius</i>	-	3.5%	Common
22.	<i>Apodemus uralensis</i>	3.9%	8.1%	Common
23.	<i>Apodemus flavicollis</i>	-	1.6%	Common
24.	<i>Clethrionomys glareolus</i>	-	1.1%	Common
25.	<i>Microtus arvalis</i>	38.9%	9.2%	Abundant
26.	<i>Microtus rossiaemeridionalis</i>	-	3.2%	Common
27.	<i>Lepus europaeus</i>	3 ind./1000 ha	2-4 ind./1000 ha	Common
28.	<i>Vulpes vulpes</i>	6 ind./1000 ha	18 ind./1000 ha	Abundant
29.	<i>Mustela nivalis</i>	2 ind./1000 ha	1-2 ind./1000 ha	Rare
30.	<i>Mustela putorius</i>	-	1-2 ind./1000 ha	Rare
31.	<i>Martes foina</i>	-	1-2 ind./1000 ha	Rare

During the study period 7 bat species were registered: Daubenton's bat (*Myotis daubentonii*), whiskered bat (*M. mystacinus*), common noctule (*Nyctalus noctula*), soprano pipistrelle (*Pipistrellus pygmaeus*), serotine bat (*Eptesicus serotinus*), parti-colored bat (*Vespertilio murinus*) and grey long-eared bat (*Plecotus austriacus*). The most abundant were the common noctule and the serotine bat that constituted more than 70% on airport territory and over 60% in adjacent ecosystems. Both species are well adapted to anthropic environment, use for hibernation, reproduction and shelter various types of buildings and hunt insects in open biotopes. The soprano pipistrelle is also well adapted to urban environment, hibernating in large colonies, breeding in buildings and hunting for insects in open areas. The Daubenton's bat was registered near the water basins outside the airport area. Other bat species were recorded in small number and were observed in the buildings near the airport, where they find shelter in the attics, cracks in the walls, empty spaces of balconies etc.

The rodent fauna was most well represented, with 14 species on airport territory and in adjacent ecosystems (tab. 1). The mole rat was registered all over the studied territory with the density of 1-2 ind./ha on the territory and of 2-3 ind./ha in surroundings. Tree associations present in small amount on the airport territory and rather abundant in adjacent areas create favorable conditions for squirrel and hazel dormouse,

the last one being rarely recorded only in adjacent arboreous vegetation. The synanthropic species *R. norvegicus* and *M. musculus* were registered near various buildings and water basins from the airport territory with more intense anthropic activity and accumulation of household waste. The species *Microtus rossiaemeridionalis* was registered in black locust stands outside airport territory.

The dominant species the small rodent community at the airport was the wood mouse (*A. sylvaticus*) with more than 50%, registered mostly near the perimeter and in bush vegetation. It is a eurytopic species that inhabit both forest and open ecosystems, well adapted to the anthropogenic landscape. The field vole constituted about 39% in trap assessment, however numerous colonies were found in grassland biotopes with an average density of 10-15 col./ha (up to 25 col./ha near the perimeter and 5-6 col./ha in the central part of the airport). *A. uralensis* registered 4%, followed by the *Mus* species, of which *M. musculus* (2.9%) was caught near the buildings and *M. spicilegus* (1.9%) near the perimeter, limiting with agroecosystems, where its mounds were observed.

The biotopic distribution of the most abundant small rodent species was assessed in the airport grassland and in the adjacent biotopes (fig. 2). In grassland the dominant species was *M. arvalis* with over 60%, followed by *A. sylvaticus* with

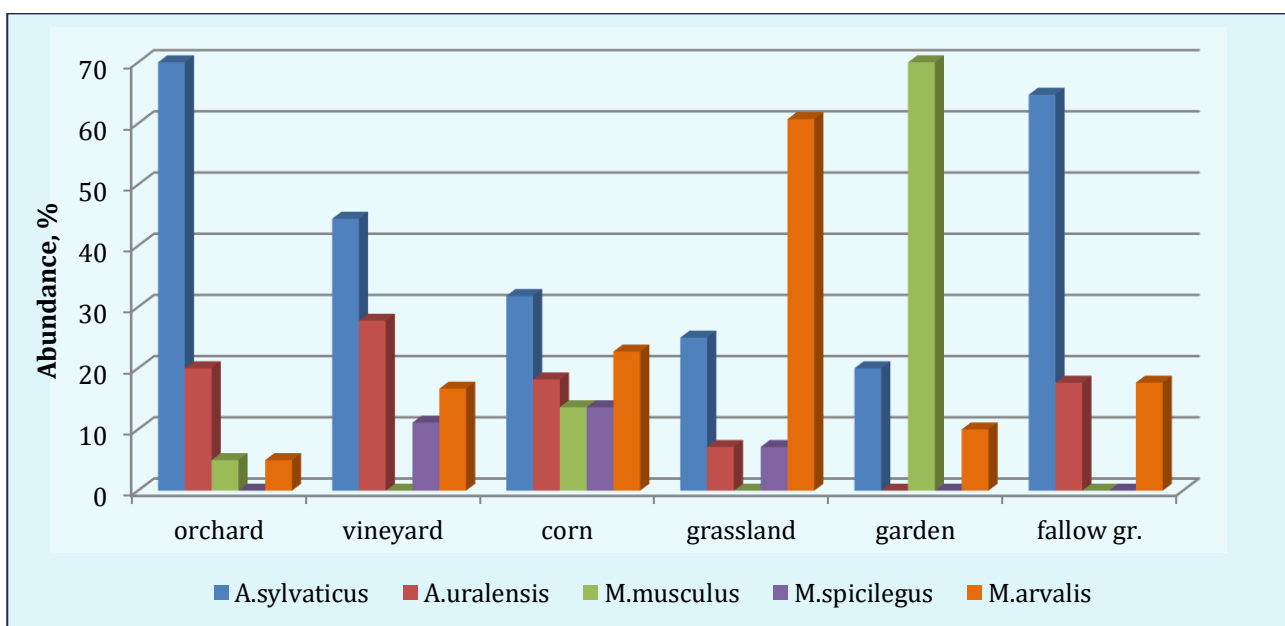


Figure 2. Biotopic distribution of the main small rodent species in airport and in adjacent biotopes.



25%, while other species had a low abundance. In the adjacent biotopes (orchard, vineyard, corn and fallow ground) the dominant species was *A. sylvaticus*, with more than 60% in orchard and fallow ground. The most even species distribution was registered in cornfield, where all 5 species were found. In people's gardens the house mouse dominated with 70%, while other 2 recorded species had much lower abundance.

The presence of cultivated lands adjacent to the airport with various crops, such as corn, sunflower, alfalfa, cereals, vineyards, orchards, as well as fallow ground is favorable for the rodent species. The presence of different buildings, waste at the airport, as well as the proximity of settlements create favorable conditions for the synanthropic species – house mouse and brown rat. Wood associations represented by decorative trees, rows of trees, forest belts, forest parks are present in small numbers at the airport and abundantly in the surrounding areas, thus creating favorable conditions for squirrels, dormouse, yellow-necked mouse and bank vole. In the humid habitats adjacent to the airport the water vole and the brown rat were recorded.

The European hare was observed several times in the sectors adjacent to the airport in fallow ground, orchards and vineyards, where this species finds favorable trophic and shelter conditions. It enters the airport through the holes under the fence, where it feeds on grassy vegetation, and the low disturbance factor is favorable. During the study period, the remnants of some hares (3 individuals) were observed not far from the runway, probably eaten by fox.

The carnivorous mammals were represented by 4 species: fox, weasel, polecat and stone marten, of which 2 species (fox and weasel) were observed on the airport territory. The weasel was observed only once in the south-eastern part, near the closest locality, and its excrements were found several times during counting routes along the southern perimeter of the airport. The fox was the most numerous and its density was of 6 ind./ha in the airport and about 20 ind./ha in adjacent biotopes (tab. 1). Traces of the fox trophic activity (hare and chicken carcasses) were found at 25-30 m from the runway in the south-eastern and southern parts of the airport, near to cultivated lands and to the localities. Even after catching the prey in the nearest ecosystems, the fox prefers to eat it

in the airport area, where there is a low level of disturbance and easy access ways.

The polecat (*M. putorius*) and the stone marten (*M. foina*) and their activity traces were seldom observed outside of the airport area in the eastern and southern parts, closer to the localities. They often inhabit rural environment and are considered anthropophilous species.

The hare and carnivorous mammals were crossing easily the perimeter of the airport due to the unproper installation of the fence at a height of 5-10 cm from the ground level. During the summer period of 2013, several dozen holes were found under the fence along the perimeter of the airport, mainly in its western and northwestern parts. On a perimeter section of about 500 m along the fence 12 old holes and 7 freshly dug ones were counted. The holes were regularly covered with earth by airport workers, but new ones appeared in the next few days. The holes were dug by the fox, but the hare and the weasel also used them to pass on the airport territory.

In the western part of the airport, mounds of sand and gravel were found, with a height of 1-1.5 m, surrounded by dense and high ruderal vegetation. In these embankments, fox burrows with several entrances were found, with many trophic remnants and excrement nearby (fig. 3). Also, in the south-western part, close to a fallow ground, other fox burrows were observed.

In an abandoned building from the central part of the airport little owl (*Athene noctua*) pellets were found. It is a sedentary species that use for food a large number of rodents in the autumn-winter period. Its presence on the territory is due to a low disturbance factor, the abundance of food objects and the presence of abandoned buildings. The analysis of pellets revealed that the trophic spectrum of the owl in the autumn-winter period consists mainly of small rodents and of insects (fig. 4).

The dominant species in little owl's diet was the house mouse, due to its rather high abundance on the airport territory and adjacent localities. The second preferred prey was the field vole with about 24%, followed by insects with 17%. The *Apodemus* genus species were found in low proportion.

Among the mammals registered on the airport territory and adjacent ecosystems most of the species were common or numerous, especially



Figure 3. Mounds of sand and gravel with several fox burrows and a fox burrow in the south-western part of the airport.

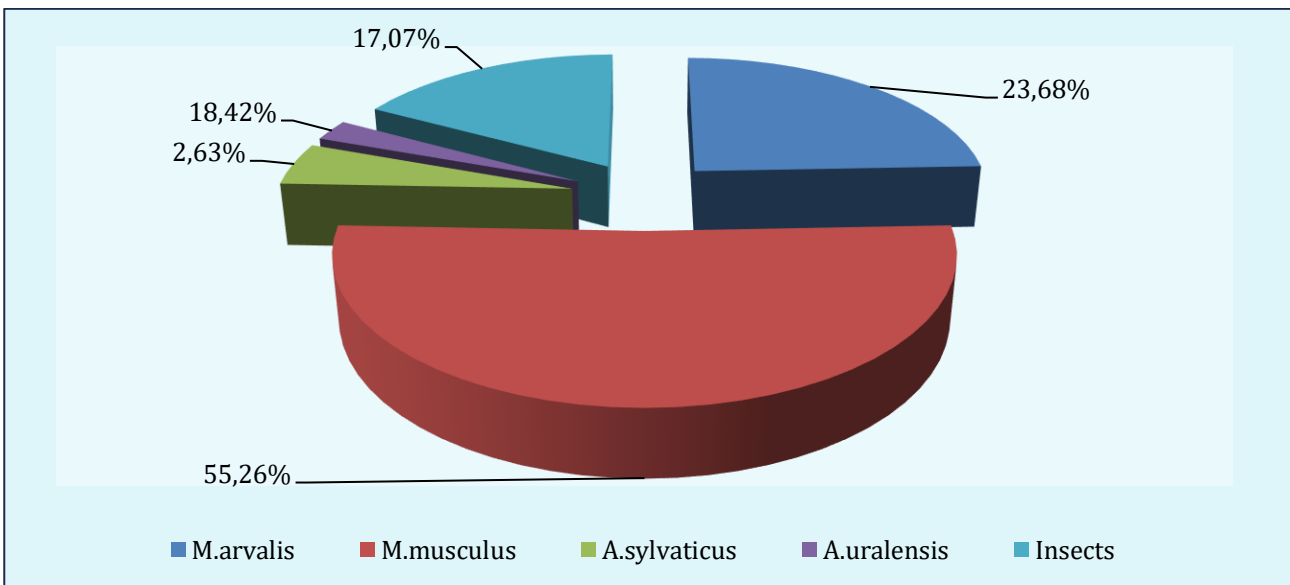


Figure 4. Trophic spectrum of *A. noctua* in the Chisinau airport.

the small rodents and the fox. 7 rare and 5 species listed in the Red Book of the Republic of Moldova were registered – *Crocidura leucodon*, *Myotis daubentonii*, *M. mystacinus*, *Vespertilio murinus* and *Plecotus austriacus* (11).

**DISCUSSIONS**

The increasing trend of wildlife strikes recorded worldwide in recent years poses a serious threat to air traffic safety (12). Among the factors responsible for this trend is the air traffic increase on a global scale, but also other factors may contribute to this increase, such as larger populations of synanthropic species or the presence of attractive sites near airports, such as landfills and fish culture ponds (1). In the case of Chisinau airport,

the adjacent sites are represented by various types of agricultural ecosystems, forest belts and parks that are attractive for many vertebrate species. Besides, over the last years large populations of mammal species have adapted to anthropic environment, which led to the increased activity of wildlife within urban settlements. Therefore, in the last decades in urban ecosystems of Chisinau city there were registered 7 insectivore species (13), 11 bat species (14), 16 rodent species and 5 carnivorous species (15, 16). Among carnivorous mammals the fox shows an increased adaptive potential for anthropogenic conditions and was frequently recorded in localities, including Chisinau city and the suburbs, where it finds favorable shelter and trophic conditions (17).



About 97% of wildlife strikes to aircraft occur with bird species, but researchers have established that terrestrial mammals and even reptiles can pose a significant risk due to their size and weight (2, 18, 19). Although, terrestrial mammals represent only 2.3% of wildlife incidents, 59% of these incidents caused damage to aircraft and almost half of the planes destroyed in wildlife incidents from 1990 to 2010 were damaged by mammals (18).

Most collisions with terrestrial mammals occur inside the airport, usually with species that normally benefit from buildings, airport structures, or the local environment. Cases of aircraft strikes with bats were not registered in Chisinau airport and bat strikes are currently considered to be a low proportion of all wildlife collisions. However, in the United States, bat strike reports have steadily increased from 4 in 1990 to a total of 255 in 2014 (18), while in some regions of Europe the estimated rate of bat collisions with aircraft is low (20).

The most serious hazard posed by small sized mammal species, especially by rodent population at airports, is the indirect risk of attracting predatory vertebrates. The rodents are the most common and eurytopic species among mammals and they serve as a food source for many species of birds of prey, carnivorous mammals and reptiles, representing an important link in the trophic chains of the living world. Among predatory groups, the prey birds pose one of the most hazardous groups of birds at the airport setting (21). In the last decades birds are considered as a threat for aircraft flights, due to increased traffic and rather high number of collisions that lead to numerous accidents (22). The aircraft size and speed increased, the noise produced by the engines decreased, thus it became more difficult for the birds to coordinate their flight, to timely detect the approach of aircraft and to avoid collision.

In the study period prey birds were often observed on the airport territory, even on the runway and on various heights (in the perimeter fence, on various buildings, on pillars) waiting for their preferred prey – the rodents. Among prey bird species the common buzzard (*Buteo buteo*), the common kestrel (*Falco tinnunculus*), the sparrowhawk (*Accipiter nisus*) and the little owl (*Athene noctua*) were registered more frequent. For most of them the main trophic objects are the

rodents, especially the field vole, which exhibit multi-year cycles and reach population peaks every 3-5 years. Many prey birds are attracted to areas such as airports during the peaks of these population cycles (5).

For the nocturnal prey birds, the *Microtus* voles are usually the preferred prey, but in the diet of little owl a high proportion constitute the insects and other invertebrates (23 - 28). The contribution to the main prey categories of little owl diet usually vary seasonally, in the spring-autumn period invertebrates were more frequently preyed, while the rodents dominated in winter (29, 30). In Chisinau airport, the main rodent prey of *A. noctua* was the house mouse, due to highly anthropized territory and proximity of localities.

One of the most important myophagous (mouse eating) mammal species is the fox, frequently recorded in the airport. It is an eurytopic species, which has increased number in various types of ecosystems, including localities. In 2008-2015 the density of the fox was extremely high and exceeded the ecological norm of about 10 times, being registered with an abundance of 18-21 ind./1000 ha in various ecosystems of the republic (17). The fox finds favorable living conditions on agrocoenoses adjacent to the airport (gardens, corn, fallow ground), enters the territory in search of food and because of the low anthropogenic disturbance. Bodies of water and natural vegetation present in airport vicinity can act as a refuge for foxes (2, 3). Several times during winter, the airport staff observed the fox running to the runway immediately before the take-off or after the landing of the planes, where it lied down on still warm runway.

At the end of the 2-year period of airport fauna monitoring several measures were recommended in order to reduce the density and abundance of mammal species that pose a risk for aircraft safety:

- Regular perimeter control. Burying the fence around the airport perimeter 10-15 cm into the ground or building a concrete foundation.
- Removal of existing sand, gravel or earth mounds, removal of various embankments and avoid waste accumulations.
- Installation of gratings in all hatches, drain pipes and channels leading outside the perimeter of the airport.

- Periodic mowing of the grass cover throughout the territory, the optimal height is 5-10 cm.
- When possible, removal of shrubs and ornamental trees.
- Removal of unused buildings, periodic control of attics, collecting of bats and owls that breed and/or spent the winter there and their release outside the airport territory (since these are useful and sometimes rare species).
- Annual availability of a forecast of the rodent number; periodic treatment with rodenticides, mandatory in the spring and autumn periods, and in the peak years up to 4 times a year.
- Regular monitoring of the entire territory to detect fox burrows and their destruction, burying the holes under the perimeter fence.
- Periodic treatment with insecticides in spring and summer periods to avoid insect breeding that attract bats and birds.

- Avoiding cereal grain cultivation in the adjacent to the airport sectors as these crops are attractive for rodents as well as for grain-eating birds.

Following our recommendations, the holes under the fence were regularly covered with soil by the airport workers, new holes appeared more and more rare and by the end of 2014 their number decreased significantly. Also, after the removal of soil and sand mounds no more fox activity was registered in the sector.

Aircraft – wildlife strikes present a global issue requiring both a local and global analytical perspective. Regional characteristics must be considered when planning airports and managing airport wildlife, especially in areas with high biodiversity (19).

## CONCLUSIONS

1. The mammal fauna of Chisinau airport was represented by 31 species – 5 insectivores, 7 bat, 14 rodent, 1 lagomorph, 4 carnivorous species. Most of the mammal species were common or numerous, 7 were rare and 5 species listed in the Red Book of the Republic of Moldova – the bicour shrew and 4 species of bats.
2. The most abundant and widespread species were 5 rodent species (*Microtus arvalis*, *Apodemus sylvaticus*, *Apodemus uralensis*, *Mus musculus*, *Mus spicilegus*) and the fox. On airport territory the most abundant was the field vole, with an average density of 10-15 col./ha, and in adjacent biotopes the wood mouse the most abundant. The fox was the most numerous carnivorous species and its density reached 6 ind./ha in the airport and about 18 ind./ha in adjacent biotopes.
3. The dominant species in little owl's diet was the house mouse with 55%, followed by the field vole with about 24%, and insects with 17%. The *Apodemus* genus species were found in low proportion.
4. The higher risk for aircraft safety is posed by rodents species that are attractive for prey birds, and by medium sized carnivorous, such as the fox, which can provoke direct damage to the aircraft.
5. Long-term recommendations were developed in order to improve the aircraft flight safety in Chisinau airport.

## CONFLICT OF INTERESTS

No conflict of interests.

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- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**

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## Conceptul *One Health*

Sănătatea umană



OMS a definit în 1946 sănătatea ca fiind „o stare pe deplin favorabilă atât fizic, mintal cât și social, și nu doar absența bolilor sau a infirmităților”, cu o completare ulterioară „capacitatea de a duce o viață productivă social și economic”.

Sănătatea animală



OIE definește bunăstarea animalelor în 2008: un animal este în bună stare dacă este sănătos, se bucură de confort, este bine hrănit, se află în siguranță, poate să își manifeste comportamentul înăscut (natural) și nu suferă din cauza unor stări neplăcute, precum durere, frică și stres.

Sănătatea plantelor  
și mediului



Sănătatea mediului se referă la acele aspecte ale sănătății umane ce includ calitatea vieții determinată de factorii fizici, biologici, socio economici și psiho sociali din mediul ambiant. Interrelațiile omului cu mediul preocupă medicina, atunci când un sistem ecologic este în stare de echilibru, prevalează starea de sănătate a populației.

La nivel global conceptul *One Health* este o strategie mondială de extindere a colaborărilor interdisciplinare și a comunicărilor în toate aspectele legate de îngrijirea sănătății oamenilor, animalelor domestice sau a faunei sălbatice, care nu mai poate fi abordată separat ci doar în comun.

*One Health* se referă nu numai la preocupările legate de bolile ce apar la oameni și animale, ci și la aspecte legate de stilul de viață, dietă, exercițiu, impactul diferitelor tipuri de relații om-animal și expuneri de mediu care pot afecta ambele categorii populaționale. Pentru a se atinge efectele scontate este nevoie și de o educație a populației care să conștientizeze factorii de risc și beneficiile prevenției, dar și de comunicare și înțelegere între pacienți și furnizorii de servicii de sănătate.

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